Extended morning fasting, energy balance and human health

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EXTENDED MORNING FASTING, ENERGY BALANCE AND HUMAN HEALTH

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Abstract

Cross-sectional evidence associates breakfast omission with negative health outcomes. The present work aimed to examine if these cross-sectional associations have a causal component, by conducting randomised control trials in healthy humans. It was established using lean individuals that there are divergent hormonal responses to morning feeding and fasting, although increased energy intake at lunch following fasting incompletely compensated for breakfast intake. Hormonal and subjective appetite responses in the afternoon did not consistently provide evidence for increased hunger following fasting. In the same participants assigned to a 6-week free-living intervention of either 700 kcal pre 11:00 or fasting until 12:00 daily, it was found that energy intake was greater in those assigned breakfast consumption, but that physical activity was also greater than those fasting. Cardiovascular risk factors and measures of metabolic control were largely unaffected by either intervention. There was no adaptation of acute metabolic/hormonal responses to feeding following either intervention. In obese individuals, similar patterns of results were obtained for the hormonal and metabolic responses to acute feeding and fasting, but with no compensation for breakfast intake at lunch. Results from the free-living intervention demonstrated no difference in energy intake between groups or physical activity over the entire day, but greater energy expenditure during the morning in those consuming breakfast. Markers of cardiovascular health and metabolic control were generally not differently affected by either intervention. Neither intervention caused adaptation of the acute hormonal and metabolic responses to feeding. In summary, acute morning fasting does not cause complete compensation for breakfast intake at lunch, or result in greater hunger throughout the afternoon. Daily morning fasting does not affect acute responses to feeding or cause increased energy intake or weight gain relative to self-selected breakfast consumption, but seems to limit physical activity in lean, and to a lesser extent, in obese individuals.

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Abbreviations

AEE: Activity Energy Expenditure
AgRP: Agouti Related Peptide
ANOVA: Analysis of Variance
ATP: Adenosine Triphosphate
AUC: Area Under Curve
A-V: Arteriovenous
BMI: Body Mass Index
BOLD: Blood Oxygen Level Dependent
CCK: Cholecystokinin
CGMS: Continuous Glucose Monitoring System
CHD: Coronary Heart Disease
CHO: Carbohydrate
CI: Confidence Interval
C-ISI: Composite Insulin Sensitivity Index
CNS: Central Nervous System
CO_2: Carbon Dioxide
CRP: C-Reactive Protein
CSFII: Continuing Survey of Food Intakes by Individuals
CV: Coefficient of Variation
DEXA: Dual Energy X-Ray Absorptiometry
DIT: Diet Induced Thermogenesis
DPP-4: Dipeptidyl Peptidase-4
EDTA: Ethylenediaminetetraacetic Acid
EE: Energy Expenditure
EI: Energy Intake
ELISA: Enzyme-Linked Immunosorbent Assay
ES: Energy Storage
FMI: Fat Mass Index
fMRI: Functional Magnetic Resonance Imaging
GOAT: Ghrelin O-acyl transferase
GLP-1: Glucagon-Like Peptide-1
GHSR1a: Growth hormone secretagogue receptor type 1a
HDL: High Density Lipoprotein
HMG-CoA: Hydroxy Methyl Glutaryl Co-A
HOMA-IR: Homeostatic Model Assessment-Insulin Resistance
HR: Heart Rate
iAUC: Incremental Area Under Curve
LDL: Low Density Lipoprotein
MET: Metabolic Equivalent
nCI: Normalised Confidence Interval
NDNS: National Diet and Nutrition Survey
NEAT: Non Exercise Activity Thermogenesis
NEFA: Non-Esterified Fatty Acids
NHANES: National Health and Nutrition Examination Survey
NHS: National Health Service
NPY: Neuropeptide Y
NTS: Solitary Tract
O_2: Oxygen
OGTT: Oral Glucose Tolerance Test
OR: Odds Ratio
PA: Physical Activity
PAEE: Physical Activity Energy Expenditure
PO_2: Partial Pressure of Oxygen
POMC: Proopiomelanocortin
PRO: Protein
PYY: Peptide Tyrosine Tyrosine
RER: Respiratory Exchange Ratio
RMR: Resting Metabolic Rate
SD: Standard Deviation
SEM: Standard Error of the Mean
SES: Socio-Economic Status
T2D: Type 2 Diabetes
TEE: Total Energy Expenditure
UK: United Kingdom
US: United States
USA: United States of America
$\dot{V}CO_2$: Carbon Dioxide Production
$\dot{V}_E$: Volume Expired
$\dot{V}_I$: Volume Inspired
$\dot{V}O_2$: Oxygen Consumption
α-MSH: α-Melanocyte-Stimulating Hormone

Chapter 1: Review of Literature

1.1 General Introduction

Obesity is a growing public health problem; it has been predicted that there will be 11 million more obese adults in the UK by 2030, with associated yearly medical costs of ~£2 billion pounds (Wang et al., 2011). While there is much debate as to whether the predominant contributor to this development is reduced physical activity (Church et al., 2011; Ladabaum et al., 2014) or greater energy intake (Swinburn et al., 2009; Westerterp and Speakman, 2008), concurrent with increased obesity it has been reported that breakfast consumption has been declining over time in US adults (Kant and Graubard, 2006). Some authors suggest that this may be a contributory factor in the increased prevalence of obesity and associated chronic diseases (Timlin and Pereira, 2007).

There have long been suggestions that increased food frequency may be associated with beneficial metabolic outcomes. As early as 1963, Gwinup and colleagues presented experimental work in humans advocating a "nibbling" pattern of eating for improved glucose tolerance and reduced serum lipids (Gwinup et al., 1963a; Gwinup et al., 1963b). Subsequently, Fabry and colleagues highlighted cross sectional associations between infrequent meal patterns and impaired glucose tolerance, hypercholesterolaemia and overweight (Fabry et al., 1964). While an infrequent meal pattern does not necessarily equate to skipping breakfast, the concept of breakfast consumption being important for health has long been entrenched in the public consciousness (Brown et al., 2013; Casazza et al., 2013). A central tenet of this belief has been based around a proliferation of associational evidence linking breakfast skipping and risk of obesity. Despite the extremely robust nature of this relationship (Figure 1.1), the evidence for causal mechanisms linking breakfast consumption with improved health is limited.

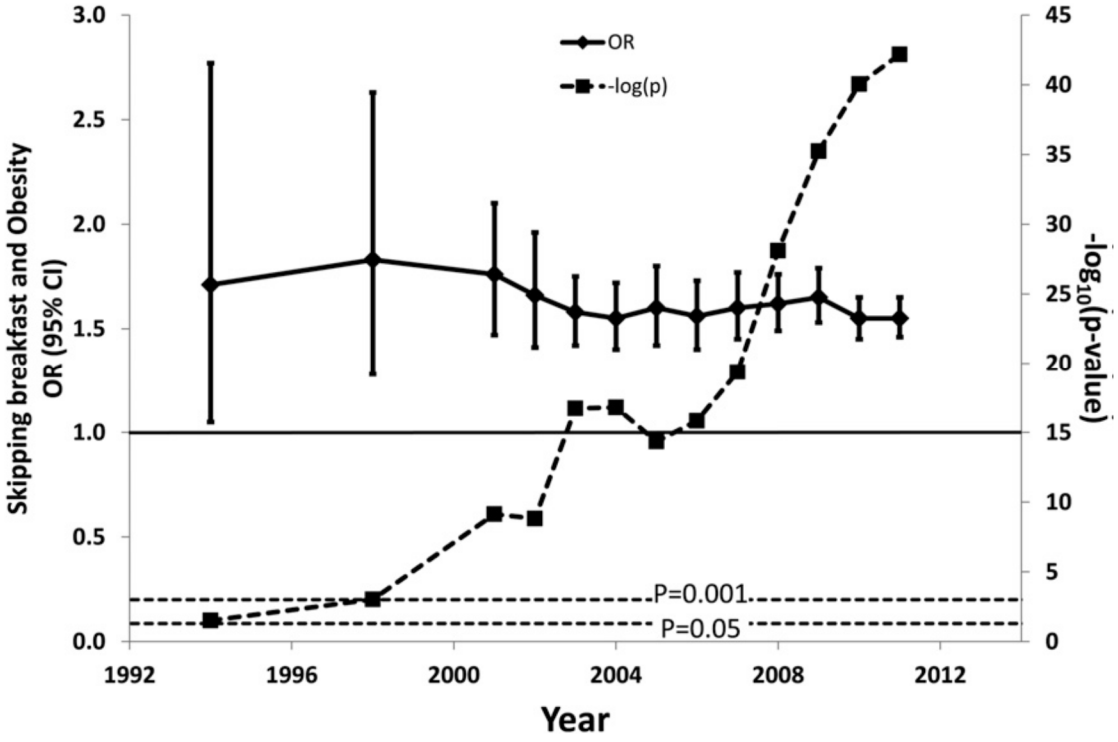

Figure 1.1: Cumulative analysis of the relationship between breakfast omission and obesity. The dashed line represents the *p* value over time, and diamonds represent the odds ratio for obesity bounded by a 95% confidence interval. Taken from Brown et al (2013). This figure illustrates that over time when studies are examined cumulatively, the odds ratio for breakfast skipping and obesity has changed very little. However, the statistical significance of this relationship has now reached $p = 10^{-42}$, indicating a very strong associational relationship.

Despite the relative lack of evidence from randomised controlled trials, breakfast consumption is strongly promoted by public health organisations. For example, amongst the 8 basic tips for healthy eating provided on the NHS choices website, one point is *"Don't skip breakfast"*. The potential implications for public health of a better understanding of the effects of breakfast consumption/omission cannot be underestimated. Unlike a number of other behaviours that are relevant for health, the decision to either consume food in the morning or extend the overnight fast is one that is taken by every individual on a daily basis. As such, the potential impact of more refined public health messages better supported by causal evidence concerning the effects of morning fasting/breakfast consumption are vast. If public health messages are to be as definitive as "Don't skip breakfast" it is crucial they are supported by a level of evidence to warrant such absolutism.

1.2 Components of Energy Balance

The first law of thermodynamics states that energy cannot be destroyed but can only be transformed from one form to another. In the context of human energy balance, this law is generally expressed as:

$$E_S = E_I - E_E$$

Where:

E_S = Energy Storage, E_I = Energy Intake and E_E = Energy Expenditure

The constituent parts contributing to energy balance will be considered below, but the net results of fluctuations of energy balance are reflected in energy storage. Energy is stored in the body as carbohydrate, fat and protein stores. Fat and carbohydrate are the primary dynamic energy storage depots within the body, but the relative contributions of both macronutrients to energy storage are vastly different. Carbohydrate storage is mostly limited to glycogen stored in muscles and the liver and is in the range of several hundred grams (Wasserman, 2009). In contrast, the storage capacity of energy in the form of stored fat is much greater and essentially infinite, with the potential for ≥1 million kcal of stored energy as triglycerides in adipocytes in a severely obese individual (Hirsch and Knittle, 1970). Therefore, as carbohydrate storage is limited, increased energy storage over an extended period of time takes the form of increased adiposity (Schrauwen, 2007).

Energy intake is solely determined by the metabolically available energy from food intake. Not all of the energy present in food is metabolically available, with the net absorption of the major macronutrients (carbohydrate, protein and fat) incomplete due to faecal losses accounting for between 2-10% of gross intake (Hall et al., 2012). The magnitude of these losses are influenced by factors such as macronutrient composition (Southgate and Durnin, 1970), fibre and resistant starch intake (Behall and Howe, 1995) and gut microbiota (Jumpertz et al., 2011). Metabolically available energy varies between macronutrients, with the most commonly used energy densities 4 kcal/g for carbohydrate and protein, 9 kcal/g for fat and 7 kcal/g for alcohol (Hall et al., 2012). The regulation of energy intake will subsequently be addressed.

Unlike energy intake, which is determined solely by dietary intake of macronutrients, energy expenditure is comprised of three components; namely resting

metabolic rate (otherwise referred to as resting energy expenditure), diet induced thermogenesis (otherwise referred to as the thermic effect of feeding) and activity energy expenditure.

Resting metabolic rate (RMR) is in a large proportion of individuals the greatest single contributor to energy expenditure (Carpenter et al., 1995). Resting metabolic rate is not consciously modifiable but is related to body mass (Ravussin et al., 1982; James et al., 1978) and in particular fat-free mass (Johnstone et al., 2005; Fukagawa et al., 1990; Cunningham, 1991; Weinsier et al., 1992), although other factors such as the high metabolic rate of the brain and other organs (Javed et al., 2010) contribute to some of the variability observed in resting metabolic rate. The factors mentioned above are generally relatively stable over short periods of time, but energy imbalance has also been suggested to cause alterations in resting metabolic rate beyond those accountable by change in body mass, referred to as adaptive thermogenesis (Dulloo et al., 2012). Decreases in mass adjusted RMR have been demonstrated in both starvation and hypocaloric dieting (Dulloo and Jacquet, 1998; Doucet et al., 2001; Martin et al., 2007). Conversely, shorter-term overfeeding (2-8 weeks) has been found to cause increased RMR beyond that predicted by weight gain (Ravussin et al., 1985c; Harris et al., 2006), although some authors suggest this may be partly attributable to persistently increased diet induced thermogenesis from the evening before regardless of overnight fasting (Joosen et al., 2005).

Diet induced thermogenesis (DIT) is the smallest component of energy expenditure in normal circumstances and reflects the obligatory energy expended for the processing and digestion of food. This is due to energy requirements of ATP hydrolysis during intestinal absorption, initial steps in metabolism and nutrient storage (Tappy, 1996). Diet induced thermogenesis is normally calculated as the energy expenditure above basal fasting level divided by the energy content of the food ingested, and is therefore expressed as a percentage (Westerterp, 2004). Different macronutrients induce varying levels of thermogenesis (Tappy, 1996; Westerterp et al., 1999), with proteins responsible for the greatest increase in expenditure (~20-30%), followed by alcohol (~10-30%), carbohydrates (~5-10%) and finally fat, which induces the least thermogenesis (~0-3%). In individuals consuming mixed diets, the generally suggested proportion of energy intake that is expended as DIT is 10%

(Westerterp, 2004). Therefore, if an individual is in energy balance, DIT would only contribute 10% towards energy expenditure, although there is substantial variation between and within individuals. It has been observed that DIT is reduced with obesity (Segal et al., 1992; Laville et al., 1993; D'Alessio et al., 1988).

Activity energy expenditure (AEE) is split into two main components, non-exercise activity thermogenesis (Levine et al., 1999) and exercise energy expenditure. Non-exercise activity thermogenesis (NEAT) was originally defined as "the activities of daily living, fidgeting, spontaneous muscle contraction and maintaining posture when not recumbent" (Levine et al., 1999). These two components combined can be referred to as physical activity (Caspersen et al., 1985). Physical activity energy expenditure is the most malleable of the components of energy expenditure (Westerterp, 2003), and the factor that is most under volitional control for the majority of individuals. Physical activity energy expenditure can account for between 5 % of total energy expenditure in the most sedentary individuals, to 70 % of total energy expenditure in endurance athletes undertaking heavy, sustained exercise (Westerterp et al., 1986). In an individual with an average level of physical activity this percentage is approximately 33% (Westerterp, 2008). Components of physical activity energy expenditure will be discussed in further detail subsequently.

Energy balance is dynamically regulated, and energy balance can only be considered when an element of time is included. Energy balance changes substantially within days (primarily due to the intake of meals) and between days due to varying dietary intake and physical activity (McKiernan et al., 2008). Despite this short-term variability in energy balance, it is energy imbalance over longer periods of time that leads to the development of excess adiposity. However, it is also possible that obese individuals can maintain long term energy balance but that this occurs at a greater level of body fat (Hall et al., 2012). If energy balance is considered over a period of a year, in individuals that change by less than one kilogram in mass, it has been suggested that energy intake has to be matched to expenditure within ~ 22 kcal/day (Hall et al., 2011). Therefore, energy balance to maintain long term weight maintenance has to be very precise.

The components of energy balance have been considered in isolation above but various components of energy balance can (and do) interact with each other. These

interactions between specific components of energy balance will be considered in greater detail when reviewing literature specific to the effect of interventions upon energy balance.

1.3 Measuring Components of Energy Balance

As discussed above there are several different components of energy balance. There are numerous different methodologies for assessing the volitional aspects of energy balance (namely physical activity energy expenditure and energy intake) which will be discussed briefly below.

1.3.1 Laboratory Energy Intake Assessment

Within the laboratory setting there are two main methodologies for assessing *ad libitum* energy intake; namely the fixed course approach and the buffet design (Blundell et al., 2010). While the buffet meal allows some assessment of food choices, it has been argued that unless food choice is the particular research question of interest, that this approach should be avoided. The rationale behind this assertion is that the provision of a wide variety of food items in a buffet scenario is at odds with the usual eating behaviour of the majority of the population (Blundell et al., 2010). It is also likely that the presence of a variety of food cues will delay satiation and promote increased food intake (Hetherington et al., 2006).

The fixed course approach involves provision of one foodstuff, which is normally comprised of a variety of ingredients combined (e.g pasta with tomato sauce) and provided *ad libitum*. This approach is beneficial when the research question is directed towards assessment of energy intake as opposed to nutrient intake (Blundell et al., 2010). It is important to consider the nature of the food provided in the single course approach, as several factors will combine to determine the amount consumed. It is important that the food provided is considered "appropriate" for consumption; this assessment of suitability is mainly based upon cultural ideals influenced by a wide range of factors (e.g geography, climate, food availability) (Furst et al., 1996). In addition to the learned acceptability of food for consumption, total energy intake is also influenced by prior learning effects relating to the expected satiating quality of foods (Brunstrom, 2007). This assessment of the expected satiety from a meal usually occurs in concert with visual cues relating to consumption volumes (e.g knowing a bowl of soup is a "normal" amount), such that covert manipulation of visual cues (secretly refilling the soup bowl as it is being eaten) results in greater consumption

volumes with no difference in perceived consumption (Wansink et al., 2005). Therefore, if the aim of the *ad libitum* meal is to attempt to reduce the influence of these prior portion expectations upon intake, then it is advisable to attempt to minimise visual feedback and an ability to complete a "portion", as in the majority of cases humans complete what is on their plate (de Graaf et al., 2005).

While it has been stated previously that the provision of a wide variety of foods in a buffet setting may delay satiation, the converse is also the case. This phenomenon is referred to as sensory specific satiety (Rolls et al., 1981a), and relates to the reduction in acceptability of a food as it is consumed and explains monotony and reduced palatability of homogenous meals (Rolls, 1986). This decrease in palatability of the specific food consumed does not result in similar reductions to other types of foods not consumed (Rolls et al., 1981b; Rolls et al., 1984). Indeed, the most common manifestation of this phenomenon in food consumption is the maintained pleasantness of sweet foods after eating savoury foods to satiety (Guinard and Brun, 1998; de Graaf et al., 1993; Griffioen-Roose et al., 2009). Therefore, as palatability affects satiation (De Graaf et al., 1999), it should be taken into account that the lack of variety (particularly the lack of contrast between sweet and savoury foods) may result in termination of feeding due to boredom rather than "true" satiety.

Therefore, as outlined above, the choice of methodology for assessing satiation should take into account the specific aims of the *ad libitum* meal and be carefully considered to maximise the appropriateness of the meal provided. In the work presented, a single course meal will be utilised as the primary outcome in the laboratory work is assessment of energy intake rather than food choices *per se*, as these aspects of food selection will be assessed during the free-living elements of the research.

1.3.2 Free-Living Energy Intake Assessment

There are several methods of attempting to establish energy intake, which require varying levels of experimenter and participant involvement. These include prospective methods where participants record synchronous with intake (i.e food diaries, both weighed and estimated) and retrospective methods that involve recall of foods consumed (usually with questioning via an experimenter). Diet records have

been shown to be more accurate in assessing macronutrient composition of diets of known composition than food frequency questionnaires (Schaefer et al., 2000). Weighed records have been shown to result in less underreporting when compared with food history interviews (Martin et al., 2002). Weighing of foodstuffs also negates the potential error in estimation of weight as Gittelsohn and colleagues (1994) have reported lower accuracy of weight estimates in foods of high volume and low weight. All methods of dietary assessment are prone to several forms of bias (Lissner et al., 1998), such as the participant changing behaviours due to greater awareness of food consumption (Macdiarmid and Blundell, 1997; Barrett-Connor, 1991), the participant deliberately omitting foods consumed due to a desire to provide socially desirable responses (Hebert et al., 1995) and the participant forgetting foods consumed (Trabulsi and Schoeller, 2001).

The result of all of these issues is that when compared with known energy intake (either via control of food provision) or when compared to energy expenditure measured via doubly labelled water reported energy intake is usually below that expected. The magnitude of this underestimation is highly variable (Hill and Davies, 2001), with factors such as gender (with females generally underreporting to a greater extent) (de Vries et al., 1994; Johnson et al., 1994) and obesity status (with obese individuals usually underreporting to a greater extent than lean) (Prentice et al., 1986; Goris et al., 2000; Lichtman et al., 1992) affecting the degree of underreporting. Despite this variability, several studies indicate an approximate underestimation of ~20% from weighted food records (De Castro, 1994b; Livingstone et al., 1990). As a result, several authors have suggested that self-reported energy intake should not be utilised for forming scientific conclusions (Dhurandhar et al., 2014b). However, it is important to recognise that when comparing experimental groups the inherent error in measurement of self-reported energy intake should be similar (De Castro, 1994b). Therefore, whilst the reported energy intake of individuals should not be used to estimate absolute measures of energy balance (i.e energy intake *versus* expenditure), estimates of energy intake can be compared (i.e comparing the self-reported intakes of the two experimental groups).

Food diaries are normally of a duration between 3 (typically 2 weekdays and 1 weekend day) and 7 days. The main reason for this is to attempt to capture some of

the difference between weekday and weekend days, as it has been observed that intake is greater on weekend days (de Castro, 1991). It has previously been suggested that the degree of misreporting increases as the recording duration increases (Young and Trulson, 1960), however, more recent work has suggested that the reduction in reported energy intake is <15 kcal per day of reporting (Whybrow et al., 2008). As there is between day variability in energy intake (Tarasuk and Beaton, 1991), this variability can be reduced by taking a mean from a longer period of recording (Borrelli, 1990) with a week of recording generally reflecting one cycle of human behaviour (de Castro, 1991), it is therefore recommended that longer duration recording periods are used (Whybrow et al., 2008). It has been suggested that one way to attempt to minimise biased reporting with food diaries and to minimise any fatigue effects is to utilise a contingent reinforcer (De Castro, 1994b). This involves informing the participants that feedback will be provided on their diet data provided, to promote faithful and thorough reporting.

It is clear that there are some inherent limitations of the self-reporting of food intake. However, with these issues considered (and in the absence of viable alternatives) a 7 day weighed food diary to compare reported energy intake between groups is an appropriate methodology.

1.3.3 Free-Living Energy Expenditure

Measurement of energy balance would not be complete without assessment of physical activity energy expenditure. In free-living situations there are several different approaches for quantifying energy expenditure, including self-report measures and various different monitoring devices that measure various parameters to attempt to estimate energy expenditure. These include devices that measure heart rate, motion and other instruments that combine multiple measurements to estimate energy expenditure. The measurement methodology that is considered the "gold standard" for estimating total energy expenditure is the use of doubly labelled water (DLW). While this methodology has been suggested to have a bias between 1 and 7% depending on the population and model used (Bluck, 2008) there are some drawbacks of this methodology. Firstly, from a practical perspective the cost of this methodology is high relative to other approaches and requires access to specialist equipment, but of greater

importance to the work in this thesis, the DLW method does not provide any information about the temporal or intensity distribution of activity. Not only is the intensity of activity relevant as certain aspects of physical activity are associated with disease risk (e.g sitting time (Katzmarzyk et al., 2009; Dunstan et al., 2005)) but as the nature of our intervention involves restriction of energy intake during a specific time period, it is particularly instructive to establish if physical activity is affected at certain times. Therefore, this eliminates methods where there is no temporal information provided with physical activity data, leaving direct measurement devices as viable options (i.e accelerometers, HR monitors and combined devices).

Of these devices there are specific limitations with both accelerometry (where there may be occasions when energy expenditure is high but acceleration is low) and heart rate (which at times may be elevated by factors other than those resulting in substantial energy expenditure such as stress and is individually highly variable). As a result of this it has been suggested that the combination of these two sources of data improves energy expenditure estimates than using either source individually (Rennie et al., 2000) and that a branched equation model combining these data sources improves these estimates further (Brage et al., 2004; Strath et al., 2005). The Actiheart device utilises this methodology and has been shown to be valid in estimating energy expenditure of low to moderate intensity activities with no substantial fixed or proportional bias (6%) relative to indirect calorimetry while used in the group calibration setting utilising basic anthropometric measurements, gender and the age of individuals (Thompson et al., 2006). Whilst some studies have identified that individual calibration (e.g exercise tests with indirect calorimetry) of these devices results in greater explanation of the variance between individuals (Brage et al., 2007) and reduces the mean bias and limits of agreement of estimates of EE (Villars et al., 2012) this was not undertaken in this work due to the additional associated participant burden (discussed further in Chapter 2).

The previous section has identified the various methodologies available to measure energy balance, with the associated limitations and strengths of the selected approaches. The next section will cover the regulation of appetite which contributes to energy intake.

1.4 Appetite Regulation

Appetite regulation is multifactorial, with energy intake influenced by both internal and external stimuli (see Figure 1.2). As well as physiological signals that can both signal hunger and satiety in the short and long term (generally referred to as homeostatic signals) (Badman and Flier, 2005) there are also other cognitive and hedonic processes that influence the control of food intake (non-homeostatic signals) (Berthoud, 2011). Focus will be on the homeostatic regulation of appetite, through tonic and episodic appetite signals.

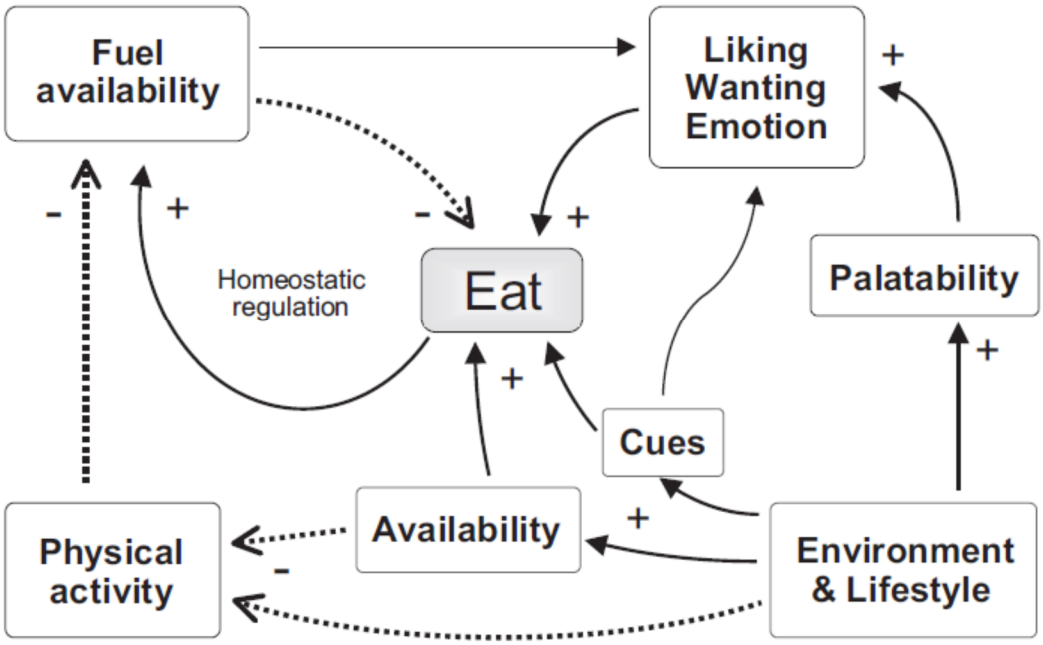

Figure 1.2: Interactions between metabolic-homeostatic and cognitive-hedonic processes in the control of food intake and energy balance taken from (Berthoud and Morrison, 2008).

As well as the regulation of the consumption of food, several different factors contribute to the regulation of satiation (the process that brings an end to eating) and satiety (processes that inhibit further eating in the postprandial period) (Blundell et al., 2010). As can be seen in Figure 1.3, these include both cognitive as well as physiological responses to feeding. The focus of the laboratory work in this thesis will be in examining elements of the post-ingestive and post-absorptive responses to feeding (in particular appetite regulating hormones), which are described in further detail in this literature review.

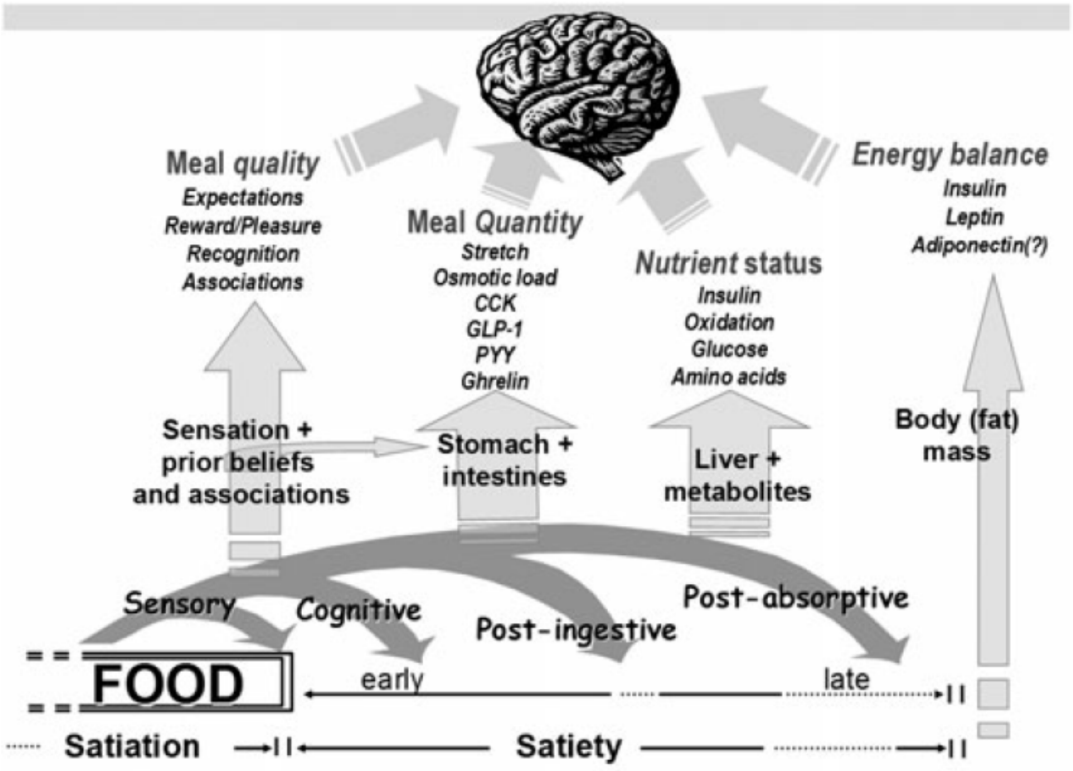

Figure 1.3: The "satiety cascade" first constructed by Blundell and colleagues and subsequently modified by Mela (2006).

1.5 Factors That Can Influence Appetite

As well as the various factors that combine to regulate eating behaviour on an individual basis as illustrated in Figures 1.2 and 1.3, research has identified differences in appetite regulation between certain groups of individuals that are outlined below.

1.5.1 Obesity

Obese individuals display delayed satiation to feeding, as energy intake prior to reaching maximum satiation is greater than lean counterparts (Delgado-Aros et al., 2004). This may be a product of a number of factors, including the greater gastric capacity of obese individuals (Geliebter, 1988) which is positively correlated with test meal intake (Geliebter et al., 1992), potentially due to reduced mechanical stretch of the stomach resulting in slower gastric emptying, inhibited CCK release and therefore delayed satiation (Geliebter et al., 1992). Additionally, several hormones implicated in regulation of appetite have been found in differing concentrations in obese individuals. Batterham et al (2003) have reported that PYY (a hormone implicated in satiety discussed in further detail subsequently) is lower postprandially in obese individuals, which le Roux and colleagues (2006) have suggested is a contributor to reduced satiety induced by meals in obese individuals. Ghrelin (an appetite stimulating hormone) concentrations are lower in obese individuals (Tschop et al., 2001b; Shiiya et al., 2002) but the feeding induced suppression of ghrelin has been reported to be lessened (le Roux et al., 2005) or abolished completely (English et al., 2002). Glucagon-like peptide-1 (a hormone implicated in glucose tolerance and satiation) release is also suggested to be reduced in obese individuals by most (Ranganath et al., 1996; Verdich et al., 2001b; Adam and Westerterp-Plantenga, 2005) but not all authors (Feinle et al., 2002).

As well as these differences in hormone responses to feeding in obese individuals, it has also been suggested that obese individuals display elevated neural responses to palatable and energy dense foods (Burger and Berner, 2014). Several authors have also suggested that obese individuals are more susceptible to environmental cues to eat than hormonal regulation (Schachter, 1968; Mela, 2001). Therefore, the combination of all the factors described suggests that both the initiation of, and responses to feeding are potentially different in obese individuals.

1.5.2 Age

It has been observed that elderly individuals do not compensate as effectively for overfeeding or underfeeding resulting in weight change as young counterparts (Roberts et al., 1994). Additionally, older adults do not reduce energy intake at an *ad libitum* feeding occasion following a snack despite greater fullness (Rolls et al., 1995). These studies have been used to propose potentially impaired energy compensation in older adults. Beyond this suggested general imprecision in compensating for deviations from energy balance, older adults (above 65 y) display reductions in body weight, even in those that were previously of healthy weight (Steen, 1988), with one of the potential explanations for this phenomenon termed the "anorexia of aging" (Morley, 1997; Morley and Silver, 1988).

Anorexia of aging refers to the decrease in energy intake and appetite associated with aging (Hays and Roberts, 2006), with several contributors including social (e.g isolation), psychological (e.g depression) and physiological factors thought to be involved (Atalayer and Astbury, 2013; Morley, 1997; Hays and Roberts, 2006). Of particular relevance to the work in this thesis, physiological regulation of appetite has been shown to be different in older individuals. Older adults report greater satiation after consumption of preloads (Sturm et al., 2004), consume less at test meals than younger individuals (MacIntosh et al., 2001; Rolls et al., 1995), with lesser hunger and desire to eat after a fixed meal in older individuals, despite no difference in fullness (Clarkston et al., 1997). There have been numerous hormonal differences that have been suggested may contribute to these findings (Moss et al., 2012), with greater CCK concentrations in older than young individuals (MacIntosh et al., 1999; MacIntosh et al., 2001), which may contribute to the observed slower gastric emptying time in older individuals (Horowitz et al., 1984; MacIntosh et al., 1999; Clarkston et al., 1997). It has also been postulated that the balance between hormones that stimulate (i.e ghrelin) and suppress hunger (i.e leptin) (Di Francesco et al., 2006) is biased towards those that suppress energy intake in elderly individuals. This hypothesis is supported by reports of greater leptin (Di Francesco et al., 2006) and lower ghrelin concentrations (Rigamonti et al., 2002; Di Francesco et al., 2008) in elderly individuals.

As there are numerous differences in the physiological responses to feeding as well as other salient factors that may affect free-living appetite differently in older individuals, in the present work individuals above 60 years of age will not be included in the experimental studies conducted. This is also appropriate as in the majority of the adult age range, focus is upon prevention of positive energy balance leading to obesity. However, in elderly individuals due to the potential problems arising from negative energy balance related to the anorexia of aging, positive energy balance is a desirable outcome in many older adults.

1.5.3 Gender

Gender differences in food intake and selection appear from adolescence, with men consuming more than women (Rolls et al., 1991). Females at equivalent normal BMI have greater levels of adiposity than males (Lovejoy et al., 2009; Gallagher et al., 1996) and in the US are reported to be approximately twofold more susceptible to severe and morbid obesity than males (Flegal et al., 2010). Accordingly, there has been some interest in differences in appetite regulation between men and women.

Physiological differences between males and females include slower gastric emptying in females (Hutson et al., 1989; Knight et al., 1997), greater concentrations of leptin relative to fat mass (Asarian and Geary, 2013) and lack of acute (Benedict et al., 2008) and chronic responsiveness to the anorexigenic effects of insulin administration (Hallschmid et al., 2004). Differences between genders of episodic regulators of appetite are less clear, with Carroll and colleagues (2007) not identifying any differences in several hormones implicated in appetite within the first hour after a liquid meal apart from greater GLP-1 concentrations in men. Other investigators have suggested that females display greater fasting (Barkan et al., 2003; Makovey et al., 2007) and fed (Greenman et al., 2004; Hagobian et al., 2009) concentrations of ghrelin, although some authors have failed to detect these differences (Tschop et al., 2001a; Shiiya et al., 2002). Neural responses to food cues (Uher et al., 2006; Cornier et al., 2010; Geliebter et al., 2013; Frank et al., 2010) and ingestion (Del Parigi et al., 2002) have predominantly demonstrated greater neural activation in females, particularly in areas related to cognitive-affective processing regions (Geliebter et al., 2013).

Despite these differences in physiology between the genders, the relative effects of gender upon appetite regulation are not well elucidated. Davy and colleagues (2007) report that in matched males and females, acute energy compensation at an *ad libitum* lunch meal for a preload in a laboratory setting was lower by 12.5% in females, despite no differences in appetite ratings. In contrast, work in free-living adults by Cornier et al (2010) suggest that when controlled eucaloric feeding occurs that women experience greater satiety than men. Additionally, the authors suggest that once the same participants were permitted to eat *ad libitum* that males overconsumed food, whereas females ate to energy needs. Therefore, there is no consistent evidence for specific appetitive responses in females. The laboratory and free-living regulation of appetite may be influenced by different factors, which is of particular relevance due to the potential role of social pressures and dietary restraint in females (Rolls et al., 1991). The effects of these moderators of intake behaviours will be discussed in the next section.

1.5.4 Dietary Restraint

Dietary restraint was first proposed by Herman and Mack (1975) and has been defined as restricting food intake in order to control body weight. It has been suggested that dietary restraint is greater in females and that restrained eaters consume less during free-living (de Castro, 1995), with this greater restraint potentially a result of greater body shame and self-objectification in women (Fredrickson et al., 1998). The body of correlational evidence linking dietary restraint and BMI has been suggested to be overwhelmingly positive in normal weight individuals and negative in obese individuals (Johnson et al., 2012). It was initially proposed there may be a role of high dietary restraint and development of eating disorders (Polivy and Herman, 1985) and data has related level of restraint with frequency/severity of binge eating (Marcus et al., 1985; Spencer and Fremouw, 1979). It has been suggested that atypical behaviours may be displayed in restrained eaters with some suggestions of counterregulatory eating (i.e greater energy intake after a preload) in laboratory situations (Herman and Mack, 1975), but others have suggested restraint alone may not predict overeating (Bellisle et al., 2009; Yeomans and Coughlan, 2009). Counterregulatory eating may only be exhibited if a highly palatable “diet breaking” (e.g ice cream) food is used for the test meal (Martins et al., 2008). Additionally, it is contended that dietary restraint

is not homogenous (Westenhoefer, 1991; Westenhoefer et al., 1999), with rigid and flexible forms of restraint, with the rigid type more likely to elicit counterregulation (Westenhoefer et al., 1994). It therefore remains difficult to conclusively establish the effect of high levels of dietary restraint upon laboratory or free-living food intake behaviours, due to a lack of consistency of data and also difficulties in assessing dietary restraint itself (Martins et al., 2008).

The previous section has introduced a variety of different factors that may affect appetite, with some consideration of their effects. The next section will introduce some of the specific hormonal mechanism affecting appetite that will be measured in this thesis.

1.6 Hormonal Control of Appetite Regulation

The homeostatic regulation of appetite is generally split into two main signal types, long-term (tonic) signals of energy storage and the short-term (episodic) regulators of appetite (Stensel, 2010; Hagobian and Braun, 2010). There are two key tonic signals of energy status, leptin and insulin. However, episodic regulators of appetite are more numerous, with several different hormones implicated in the concerted responses to both initiate feeding and respond to consumption to bring about satiation. As illustrated in Figure 1.2, the regulation of eating occasions is influenced by a range of factors beyond simple homeostatic regulation and it has been suggested that hormonal regulation is more relevant for determining the amount consumed at each feeding occasion (Strubbe and Woods, 2004; Woods, 2009). In this regard, it is suggested that the tonic appetite hormones provide a "background tone" that modifies the efficacy of the episodic hormones that bring about satiation dependent on the energy balance/storage status of the individual (Woods and D'Alessio, 2008).

1.7 Tonic Hormones

1.7.1 Leptin

Leptin is primarily secreted by adipocytes in the white adipose tissue and was originally identified and characterised by Zheng et al (1994). Concentrations of leptin are proportionate to fat mass (Schwartz et al., 1996a; Maffei et al., 1995). Leptin responds to short term energy status, although with fasting/energy restriction the reductions in leptin are far greater than the loss in fat mass (Weigle et al., 1997), a response that strongly protects against loss of fat mass.

Leptin acts on two main neuronal populations (Elias et al., 1999) in the arcuate nucleus within the hypothalamus (Schwartz et al., 1996b), suppressing expression of the orexigenic peptides Neuropeptide Y (NPY), and agouti related peptide (AgRP). Additionally, leptin stimulates action of proopiomelanocortin (POMC) neurons (Cowley et al., 2001), where this protein precursor is cleaved to α-melanocyte-stimulating hormone (α-MSH), an anorexigenic peptide. Therefore, during times of nutrient deficiency and low leptin levels, the reduced inhibitory influence of leptin on NPY/AgRP neurons and limited activation of POMC containing neurons cause an overall increase in orexigenic signalling (Moran et al., 2006).

Leptin displays a strong diurnal rhythm, peaking in the late evening and with a nadir in the morning (Schoeller et al., 1997) and is responsive to feeding, with increases in leptin concentrations due to successive meals (Saad et al., 1998b), an effect suggested to be related to the effect of insulin on modulating leptin (Saad et al., 1998a). Administration of leptin to a deficient patient resulted in decreased appetite and body mass, highlighting a key role in appetite regulation (Farooqi et al., 1999). The reported mode of action of leptin in reducing food intake is via a reduction in meal size (Eckel et al., 1998; Kahler et al., 1998; Flynn et al., 1998), through its potentiating of the feeding inhibitory effects of both cholesystokinin (CCK) (Emond et al., 1999) and gastric nutrient loads (Emond et al., 2001).

1.7.2 Insulin

Insulin is produced by the pancreas and also acts a tonic signal of energy status. Insulin concentrations are positively correlated with adiposity, and act on similar

neuronal populations within the brain as leptin (Wynne et al., 2005), although there has been recent work to demonstrate that subpopulations of POMC neurons are responsive to either insulin or leptin (Williams et al., 2010). It has also been reported that leptin receptors are also present within pancreatic islet cells and therefore leptin overproduction may modify basal and glucose stimulated insulin production (Emilsson et al., 1997). Administration of insulin has been found to decrease food intake and body weight in mice (Brown et al., 2006), rats (Chavez et al., 1995) and men (Hallschmid et al., 2004), although the effects on reducing food intake are less potent than leptin (Belgardt and Bruning, 2010), reductions of food intake and weight can be additive with leptin (Air et al., 2002).

Insulin resistance has been suggested to attenuate the responsiveness of glucose metabolism in brain regions implicated in appetite regulation to the effects of insulin (Anthony et al., 2006). These authors suggest that this may predispose individuals to obesity due to reduced reward responses to palatable foods. More applied work has demonstrated that insulin response is negatively correlated with subsequent intake in lean individuals (Holt et al., 1996). In contrast, studies comparing lean and obese individuals this relationship was only observed in lean but not obese individuals (Speechly and Buffenstein, 2000; Verdich et al., 2001b). This pattern of results has been further reinforced by Flint and colleagues (2007) who report a similar lack of relationship between insulin and intake in obese individuals when combining the results of 6 controlled feeding studies. It therefore appears that obese individuals have a reduced sensitivity to the satiating effects of insulin, potentially due to central insensitivity to the effects of insulin as described above.

1.8 Episodic Hormones

Episodic hormones are generally split into two different categories based upon their effects upon appetite. An orexigenic hormone stimulates appetite, whereas an anorexigenic hormone suppresses appetite (Smitka et al., 2013). There is currently only one known orexigenic hormone (ghrelin) but numerous anorexigenic hormones, of which those examined in the experimental work presented are subsequently reviewed.

1.8.1 Ghrelin

Ghrelin is a hormone predominantly secreted by the stomach (Kojima et al., 1999), with the gastric oxyntic glands the most abundant source of circulating ghrelin (Karra and Batterham, 2010). It is unique amongst episodic hormones, as the only known orexigenic (appetite stimulating) hormone. The primary site of action of ghrelin is the arcuate nucleus, where ghrelin has opposite effects to that of leptin. Ghrelin stimulates the activity of neurons expressing the orexigenic peptides NPY and AgRP (Nakazato et al., 2001; Kamegai et al., 2001), and inhibits POMC neurons (Cowley et al., 2003). The two main forms of ghrelin are derived from pre-proghrelin, with the biologically active form of the hormone acylated by the enzyme ghrelin O-acyl transferase (GOAT) (Delhanty et al., 2012). The acylated form of the hormone has been suggested to have appetite regulatory properties, as it can bind to the growth hormone secretagogue receptor type 1a (GHSR1a) with des-acyl ghrelin simply a degradation byproduct without biological activities (Kojima and Kangawa, 2005). As a result, many studies measure total ghrelin (i.e acylated + des-acyl ghrelin) although recent work is beginning to suggest that des-acyl ghrelin may have distinct biological functions to acylated ghrelin (Delhanty et al., 2012).

Peripheral ghrelin administration stimulates food intake in both rats (Wren et al., 2001b; Wren et al., 2000; Tschop et al., 2000) and in humans (Wren et al., 2001a; Druce et al., 2005; Druce et al., 2006). Ghrelin concentrations are reduced in obesity (Tschop et al., 2001b; Cummings et al., 2002) but increase in response to weight loss (Cummings and Overduin, 2007; Hansen et al., 2002), although acute energy restriction (-800 $kcal \cdot d^{-1}$ for 4 days) does not increase fasting or postprandial concentrations (Doucet et al., 2004). Gastric bypass surgery has been observed to

reduce ghrelin concentrations (Beckman et al., 2010), with this reduction suggested to contribute to reduced hunger and sustained weight loss with these procedures. However, this is not a universal finding, with some authors suggesting that inconsistencies in surgical (Pournaras and le Roux, 2010) and experimental methods (Beckman et al., 2010) may explain the discrepant findings.

Ghrelin levels are reduced postprandially (Tschop et al., 2001a) and rise with fasting (Cummings et al., 2001), with authors reporting pre-prandial rises in plasma ghrelin in the absence of time cues with close overlap of temporal ghrelin and appetite profiles, suggesting a role of ghrelin in meal initiation (Cummings et al., 2004). However, there is some evidence that the pre-meal surges in ghrelin may be an anticipatory response due to the entraining of meals (Drazen et al., 2006).

Ghrelin suppression following meals is proportional to the caloric load ingested (Callahan et al., 2004), with proteins and carbohydrates having a more potent effect in suppressing ghrelin than lipids (Foster-Schubert et al., 2008). The suppression of ghrelin by carbohydrates has been shown to be biphasic, with an increase above baseline levels after approximately 3 hours (Foster-Schubert et al., 2008). There is evidence to suggest that the meal induced suppression of ghrelin is either reduced (le Roux et al., 2005) or completely absent in obese individuals (English et al., 2002).

1.8.2 Peptide Tryosine-Tyrosine

Peptide tyrosine-tyrosine (PYY) is a hormone that is secreted by L-cells in the intestine and is co-secreted with glucagon-like peptide-1 (Habib et al., 2013). There are two circulating forms of the hormone, PYY_{1-36} and PYY_{3-36} which are produced by N-terminal cleavage of PYY_{1-36} by dipeptidyl peptidase-4 (DPP-4) (Mentlein et al., 1993). PYY_{3-36} the most abundant form (Batterham et al., 2006) and is thought to be most relevant in regulating intake, with studies identifying no effect of administration of PYY_{1-36} (Sloth et al., 2007a; Sloth et al., 2007b). The effects of PYY are thought to be mediated through effects on the ARC in the hypothalamus, with PYY_{3-36} selective for Y2-receptors that inhibit NPY/AgRP neurons and therefore remove repression of adjacent melanocortin producing cells (Batterham et al., 2002). After infusion of PYY_{3-36} it has been found that functional magnetic resonance imaging (fMRI) blood oxygen level dependent (BOLD) activity in orbitofrontal cortex (an area of the brain

implied in hedonic rather than homeostatic regulation) predicted subsequent food intake (Batterham et al., 2007). The implication drawn by these researchers was that PYY enhances the discrimination of the reward value of food (Batterham et al., 2007).

Circulating levels of PYY are low and fall in the fasted state (Batterham et al., 2007), and are increased rapidly in response to nutrient ingestion (Adrian et al., 1985) remaining elevated for several hours. The elevation of PYY is proportional caloric load (le Roux et al., 2006), although the macronutrient composition of feedings can have differing effects upon PYY with some authors suggesting a key role for protein (Batterham et al., 2006) and others suggesting greater PYY stimulation after high fat relative to high carbohydrate meals (Essah et al., 2007; Cummings and Overduin, 2007). PYY has been shown to act additively with glucagon-like peptide-1_{7-36} in reducing food intake (De Silva et al., 2011; Neary et al., 2005).

Administration of PYY has been shown to bring about a dose dependent reduction in food intake in rodents, and reduce appetite and 24 hour energy intake in lean humans (Batterham et al., 2002), with further work indicating that obese humans are also responsive to the anorectic effects of administered PYY_{3-36} (Batterham et al., 2003). It has been suggested that PYY contributes to satiation, with attenuated postprandial PYY release in obese subjects associated with impaired satiety (le Roux et al., 2006) and positive correlations apparent between postprandial PYY and changes in satiety (Guo et al., 2006). "High responders" for PYY report the greatest reduction in hunger following a meal (Stoeckel et al., 2008).

1.8.3 Glucagon-like peptide-1

Glucagon-like peptide-1 (GLP-1) is one of several incretin hormones. Incretins are gut hormones that amplify the secretion of insulin in response to nutrients (Holst, 2013). GLP-1 is secreted from the L cells of the intestine in response to nutrients, and is post-translationally cleaved into the biologically active forms GLP-$1_{(7-36)}$ amide and GLP-$1_{(7-37)}$, with the majority of the circulating form in humans GLP-$1_{(7-36)}$ (Orskov et al., 1994), although this is rapidly inactivated by DPP-4 (Deacon et al., 1995), such that only 10-15% leaves the liver (Holst, 2007). While incompletely understood, it is suggested that GLP-1 exerts its effects through both peripheral afferents originating in the gut that activate central nervous system nuclei involved in satiation (Williams,

2009) and central GLP-1 expression in neurons in the solitary tract (NTS) of the medulla oblongata (Dailey and Moran, 2013).

Administration of GLP-1 in humans reduces food intake in the majority of studies (Gutzwiller et al., 1999a; Gutzwiller et al., 1999b; Verdich et al., 2001a; Flint et al., 1998), with the anorectic effect of GLP-1 also evident in obese individuals (Verdich et al., 2001a). GLP-1 concentrations are reduced in obesity (Verdich et al., 2001b; Carr et al., 2010), and are increased with weight loss (Verdich et al., 2001b). Plasma GLP-1 increases in response to feeding, but the increase elicited is relatively minor, due to the activity of DPP-4 introduced above. Within the context of the dose-response relationship observed, it is suggested that this increase is unlikely to be accountable for reduced food intake (Holst, 2013), although additive effects with PYY (Neary et al., 2005; De Silva et al., 2011) and prevention of the postprandial decline in ghrelin levels (although at supraphysiological infusion concentrations) have been observed (Hagemann et al., 2007). It is therefore possible that the effects of GLP-1 are partly due to its interaction with other appetite hormones.

1.9 Subjective Indicators of Appetite

As well as measurement of hormones that are implicated in the regulation of appetite, subjective measures are also used in research settings to assess various elements of appetite in individuals. In some instances these scales are used to assess the desire to eat a specific type of foodstuff (i.e for something salty), or as utilised in the experimental work presented, to assess perceived general states of hunger and perceived consumption (Blundell et al., 2010).

While it is important to consider the reliability and validity of self-report measures of appetite, it is also crucial to acknowledge that the validity of these measures is not contingent upon a correlation with actual intake (Hill et al., 1995). There are occasions when humans eat without hunger and *vice versa* and as such appetite measures cannot be used as a surrogate of measured intake (Mattes, 1990). With this important caveat in mind, the majority of laboratory studies have reported significant relationships between appetite ratings and subsequent energy intake at test meals (Drapeau et al., 2007; Flint et al., 2000; Hulshof et al., 1993; Parker et al., 2004).

The reliability of these measures has been examined in several studies. A study using 9 participants ingesting purely carbohydrate meals, has suggested large variability of subjective appetite response (peak and nadir) to repeated tests (Raben et al., 1995). However, the same group subsequently examined the reliability of appetite measures with greater subject numbers and a more typical test meal. Flint and colleagues (2000) have established that when repeating both fasted and mean appetite sensations over 4.5 hours to a breakfast test meal, there was no mean difference between either of the testing occasions. Correlations between fasting appetite measures were least reliable, particularly when participants had not undergone prior diet standardisation. Using the data obtained in their study, power calculations suggest for a power of 0.8 and to detect a 10mm difference in a measure, that required participant numbers may be between 8-35 dependent on the nature of the experimental design (with paired designs requiring substantially fewer participant numbers). In a more recent design, it has also been established that time averaged appetite ratings assessed for 2 hours after a milk and cereal test meal are similarly reproducible to those reported over 4.5 hours (Gonzalez et al., 2012). It has therefore been established

that subjective appetite ratings are a reliable measure that can be used to detect differences between conditions given sufficient participant numbers.

The previous sections have introduced the various hormones that are thought to affect appetite and are to be measured in this thesis, as well as the methods of measuring subjective appetite. Combined with the earlier sections examining the components of energy balance and the measurement of those components, the basic theoretical framework of the relevant factors to be measured in this thesis have been considered. Therefore, the next sections begin to assess the specific literature relating to breakfast consumption and components of energy balance and other health markers.

1.10 Background to Evidence Examining Breakfast Studies

1.10.1 Scope of Literature Used

Due to the ease of implementation of breakfast interventions in school children and the putative benefits of breakfast consumption upon cognitive function (Simeon and Grantham-McGregor, 1989; Wesnes et al., 2003), numerous studies have been conducted investigating the effect of breakfast consupmtion in children and adolescents (Hoyland et al., 2009; Rampersaud et al., 2005; Edefonti et al., 2014). However, for the purposes of this thesis, focus will be on studies in adults. The two key reasons for this are that the populations used in this work are adults, and secondly, that appetite regulation and energy balance in children is necessarily influenced by different factors. As discussed in the overview of energy balance, in the majority of situations to maintain stable weight, energy intake and expenditure have to be tightly equivalent. In children, while the fundamental tenets of the energy balance equation hold, for the purposes of growth, children and adolescents necessarily require an excess of energy intake to contribute to energy storage (Hall et al., 2012). Additionally, food choices/frequency are less self-selected in children than adults, with parental habits strongly influencing behaviours in offspring (Keski-Rahkonen et al., 2003).

1.10.2 Prevalence and Trends in Breakfast Consumption

"Breakfast" is not a universally defined term, with different definitions in use throughout the literature (Timlin and Pereira, 2007). These vary based upon energy intake of the eating occasions, the types of food eaten, the time of day at which they are consumed and the time at which they are consumed relative to waking. With such a range of factors that could contribute to the definition of breakfast consumption (both when retrospectively analysing data already obtained and when asking individuals to self-report breakfast intake) it is difficult to compare associational studies that use different definitions of what constitutes the breakfast meal. Throughout this literature review, attention will be drawn where appropriate to key differences between definitions of breakfast used by different investigators.

Gibson and Gunn (2011) analysed data from 7-day food records in 1724 adults aged 19-64 y in the British National Diet and Nutrition Survey from 2000/2001. They

defined breakfast as food consumed between 06:00 and 10:00, and report that 22 % of days in men and 20 % of days in women did not include a breakfast. However, this is the proportion of all person-days and therefore no information can be obtained about the frequency of breakfast consumption in individuals (i.e whether the proportion of days missing breakfast observed is a product of ~20 % of adults missing breakfast every day, or every adult missing breakfast approximately 20 % of the week, or as would be expected to be the case, some mixture of both possibilities). In a more recent study, Reeves and colleagues (2013) utilised an internet survey in a demographically representative sample of the UK population to establish breakfast consumption patterns. In the sample of 1066 adults, 64 % of respondents reported consuming breakfast every day, with a further 8 % reporting consuming breakfast 5-6 days of the week. Infrequent consumption (1-4 days) was reported in 22 % of individuals while the remaining 6 % never eat breakfast. What constituted a breakfast was not defined in this study, as one of the objectives was to ascertain what the respondents considered to be "breakfast". In this regard, 82 % of individuals listed one of the reasons as "first meal of the day", with less popularity for eating before a certain time (14 %) and a certain type of food (19 %). While there is some evidence for cross-sectional assessment of breakfast consumption habits in the United Kingdom, there is no published research on trends in consumption over time.

Evidence on temporal trends in breakfast consumption is available from the United States, using data from the National Health and Nutrition Examination Survey (NHANES). From NHANES in 1971-1975 to 2001-2002, the proportion of participants who classified foods as breakfast as part of a 24 h dietary recall decreased significantly (p <0.0001) from 89 ± 0.6 % to 82 ± 0.6 % (Kant and Graubard, 2006). This decrease in breakfast consumption in US adults has also been reported from 1965-1991 in 24 h diet recalls from the National Food Consumption Survey (NFCS) and the Continuing Survey of Food Intakes by Individuals (CSFII), with intake of foods and drinks reported between 05:00 and 09:00 decreasing from 86 to 75% (Haines et al., 1996). A more recent report utilising the NHANES databases confirm the general trend observed previously for decreased reporting of breakfast consumption over time from 1971 to 2010 in both sexes (Kant and Graubard, 2014). However, for the continuous NHANES data for 1999-2002 up to 2007-2010, there has been no further decrease in breakfast consumption, with some evidence for stability around 80% in

both genders (Kant and Graubard, 2014). As all of this data is from single day recalls, like the work of Gibson and Gunn (2011) it is impossible to establish the frequency of breakfast consumption amongst these individuals. However, evidence would seem to suggest that the consumption of breakfast has declined over the last 50 years.

1.11 Literature Examining Breakfast Consumption

There is much available literature examining the relationship between breakfast consumption and weight/other markers of health. This evidence will be split according to the research designs employed, with three main strands of evidence examined: cross-sectional associations, prospective/longitudinal designs and finally, randomised controlled trials. As stated previously, the evidence presented will be mainly from studies in adults but where insufficient evidence exists in this population, relevant studies conducted in adolescents will be introduced for context.

1.11.1 Cross-Sectional Studies

Cross-sectional studies can provide information as to whether an association exists between two factors, but cannot be used to confirm the existence or direction of causality between those two factors. The most rigorously established of the associations between breakfast consumption exists with overweight/obesity. Whilst there has been much work completed in children examining this relationship, there are still copious numbers of studies that have examined this relationship in adults. A summary of recent studies examining this effect have been included in Table 1.1.

Table 1.1: Cross sectional associations between breakfast consumption/omission and obesity

Author	Study Population	Definition of Breakfast/Skipping	Adjustment for Confounders	Outcomes
(Azadbakht et al., 2013)	Iran 411 women 18-28 y	Food or drink before 10:00 h in >5 d per week, <5 d counted as skipper.	None	20% prevalence of overweight/obesity amongst skippers, 0.5% amongst consumers.
(Barr et al., 2013)	Canada 19913 both genders >19 y	Any foods/beverages defined as breakfast by participants for 24 h recall	Numerous including PA, diet, smoking, demographic factors	BMI greater amongst skippers relative to relative to ready to eat cereal consumers (but not other breakfast consumers).
(Berteus Forslund et al., 2002)	Sweden 83 obese women 94 lean women 37-60 y	Participant defined meal consumption between 06:00 h and 09:59 h	None	No difference in breakfast frequency between lean and obese women. Greater proportion of meals consumed at breakfast in lean women.
(Cho et al., 2003)	USA 16452 both genders > 18 y	Participant defined as "breakfast" for 24 h recall	Numerous including PA, diet, smoking, alcohol	Greater BMI in breakfast skippers but only relative to cooked cereal, ready to eat cereal and quick breads. Not different to other breakfast types.
(Deshmukh-Taskar et al., 2013)	USA 5316 both genders 20-39 y	Self-reported breakfast consumption in 24 h recall	Numerous including PA, diet, smoking, demographic factors	Weight/BMI greater in breakfast skippers, but only than those consuming ready to eat cereal, not those consuming other breakfast types.
(Huang et al., 2010)	Taiwan 15340 both genders 18-64 y	Respondents who answered question "Typically, how many days a week do you eat breakfast?" as 1 or 0 classified as skippers.	Age, sex, exercise habits, smoking, demographic factors.	Prevalence of obesity (BMI>27) greater in breakfast skipping. OR (1.34, 1.15-1.56) when controlling for confounders. Prevalence rate of obesity decreased as breakfast consumption frequency increased.

Table 1.1 continued: Cross sectional associations between breakfast consumption/omission and obesity

Author	Study Population	Definition of Breakfast/Skipping	Adjustment for Confounders	Outcomes
(Horikawa et al., 2011)	Asia Pacific Region 18+, 4 different studies	Variable, but skipping generally defined as <1 breakfast occasions per week	Limited adjustment for confounders, majority controlled for gender.	Pooled OR from adult studies in region for those that skipped breakfast and the risk of overweight/obesity was 1.93 (1.22-3.06).
(Ma et al., 2003)	USA 499 both genders 20-70 y	75 % of repeated (10-15) diet recalls to include self- reported breakfast intake	Age, gender, educational level, total EI, physical activity	OR for obesity with breakfast skipping 4.50 (1.57, 12.90), but very limited numbers classified as skippers (18:481).
(Marin-Guerrero et al., 2008)	Spain 34974 both genders 25-64 y	Response to interview question of whether breakfast was eaten or not eaten	Age, physical activity, smoking, alcohol intake, demographic factors.	OR for risk of obesity was 1.58 (1.29-1.93) in men and 1.53 (1.15-2.03) in women skippers when controlling for confounders.
(Mekary et al., 2012)	USA 29206 men 40-75 y	Reporting eating anything in the morning in response to questionnaire.	None	Greater BMI in those that skipped breakfast relative to those who consumed breakfast.
(Mekary et al., 2013)	USA 46289 women 30-55 y	Regular-Report 7 days per week breakfast consumption Irregular-Between 0-6 days.	None	Greater BMI in irregular breakfast consumers relative to regular breakfast consumers.
(Merten et al., 2009)	USA 7788 both genders 18-26 y	Response to interview, > 4 days a week breakfast consumption defined as regular breakfast, < 4 as no breakfast	Demographic variables (e.g poverty, parental presence), race and sex	OR for obesity lower in those that ate breakfast 0.41 (0.34, 0.48) for those that ate breakfast in adolescence and adulthood vs skippers, 0.77 (0.64-0.94) for those who ate breakfast in adulthood vs skippers

Table 1.1 continued: Cross sectional associations between breakfast consumption/omission and obesity

Author	Study Population	Definition of Breakfast/Skipping	Adjustment for Confounders	Outcomes
(Mills et al., 2011)	USA 1,099 women 40-60 y	Presence of food labelled as breakfast in 24 h food record	Physical activity, age, income, marital status, education, energy intake	OR for risk of overweight/obesity not different in those eating breakfast 0.74 (0.35-1.54). Suggested low proportion of skippers (4%) limits sensitivity to effect.
(Mostad et al., 2014)	Norway 50, 339 both genders > 19 y	Frequency of breakfast consumption-response to questionnaire	Age, sex	Individuals in highest quartile of waist:hip ratio had 5% less breakfast consumption than those in lowest waist:hip ratio quartile
(Odegaard et al., 2013)	USA 3598 both genders 25-37 y	Self-report breakfast consumption frequency (0-3, 4-6 and 7 d per week)	None	Stepwise increase in BMI with reduced reported frequency of breakfast consumption.
(Smith et al., 2010)	Australia 2184 both genders 26-36 y	No foods indicated between 06:00 h and 09:00 h for 24 h meal frequency recall	None	Individuals who skipped breakfast in both adulthood and as a child had a significantly greater BMI than those who had always consumed breakfast.

The studies presented in Table 1.1 conclusively demonstrate that cross-sectional associations between skipping breakfast and risk of obesity/overweight are apparent. These associations are present regardless of the various definitions of breakfast/skipping and the range of comparisons made (i.e different contrasts based upon simple yes/no responses, specific extremes of frequency of consumption and other intermediates). The associations are also apparent in several countries as well as different age and gender groups. Indeed, the relationship between breakfast skipping and obesity has been described as "gratuitously established" (Brown et al., 2013). In conducting a cumulative meta-analysis of this relationship over time, Brown and colleagues (2013) report that in studies including adults and children sourced from 4 different systematic reviews, the strength of this relationship was $p = 10^{-42}$ by 2011. However, despite the strength of this particular association, this adds no further value in establishing whether the relationship is causal in nature. This is particularly relevant as numerous studies have also reported that breakfast skipping is also associated with other negative health behaviours and demographic characteristics that are independently associated with obesity and poorer health (Table 1.2).

Table 1.2: Associations between breakfast skipping and other lifestyle and demographic factors

Greater Smoking	Lower Physical Activity	Greater Alcohol Intake	Lower Diet Adequacy	Lower SES
(Barr et al., 2013) (Cahill et al., 2013b) (Keski-Rahkonen et al., 2003) (Mekary et al., 2012) (Mekary et al., 2013) (Odegaard et al., 2013) (Purslow et al., 2008) (Smith et al., 2013) (van der Heijden et al., 2007)	(Barr et al., 2013) (Cahill et al., 2013b) (Mekary et al., 2012) (Mekary et al., 2013) (Odegaard et al., 2013) (Smith et al., 2013) (van der Heijden et al., 2007) (Wyatt et al., 2002)	(Cahill et al., 2013b) (Huang et al., 2010) (Keski-Rahkonen et al., 2003) (Mekary et al., 2012) (Mekary et al., 2013) (Odegaard et al., 2013) (van der Heijden et al., 2007)	(Azadbakht et al., 2013) (Deshmukh-Taskar et al., 2010b) (Mekary et al., 2012) (Mekary et al., 2013) (Nicklas et al., 1998) (Odegaard et al., 2013)	(Hulshof et al., 2003) (Keski-Rahkonen et al., 2003)

From an energy balance perspective, some cross-sectional studies have reported whether estimated physical activity levels are greater or lesser in individuals who skip breakfast. Several of these studies report greater levels of physical activity in those individuals classified as breakfast consumers (Barr et al., 2013; Cahill et al., 2013b; Mekary et al., 2013; Mekary et al., 2012; Smith et al., 2013; van der Heijden et al., 2007; Wyatt et al., 2002). However, all of these studies have used a physical activity questionnaire to do so, which limits their effectiveness due to their limited reliability and validity (Shephard, 2003).

On the other side of the energy balance equation, several studies have also reported relationships between breakfast skipping and energy intake. Of those studies that have related breakfast skipping or consumption to energy intake, an inconsistent relationship is apparent. The majority of studies that have simply examined whether individuals consumed more total calories when reporting skipping breakfast have reported significantly lower energy intake in those that skip breakfast (Cho et al., 2003; Deshmukh-Taskar et al., 2010a; Nicklas et al., 1998), although this has not been the case in all studies (Mekary et al., 2012). Two studies that have split breakfast consumers on the basis of breakfast consumption frequency into regular and irregular consumers, have reported that when defining irregular consumers as any less than daily (Mekary et al., 2013) or <4 days a week (Wyatt et al., 2002) there was no difference in energy intake between the two groups. Similarly, Odegaard and colleagues (2013) when examining varying frequencies of weekly breakfast consumption have not reported any difference in energy intake between individuals reporting consuming breakfast 0-3, 4-6 or 7 days a week. Two further studies (Schusdziarra et al., 2011; Purslow et al., 2008) have examined in breakfast consumers whether total daily energy intake increases concurrently with breakfast energy intake. Both of these studies conclude that as breakfast energy intake increases, total energy intake also does so. This cross-sectional evidence suggests that energy intake may potentially be reduced in those that skip/consume less energy dense breakfasts, with no evidence in adults to suggest breakfast consumers have lower energy intake. Therefore, for breakfast consumption to not result in positive energy balance relative to skipping, energy expenditure would have to be greater, an association which has been observed in some studies.

1.11.2 Prospective Studies

Whilst cross-sectional studies are a useful basis upon which to establish associations between variables, prospective designs are beneficial to establish the occurrence of clinical events/development of disease states over time in the same individuals. Within the context of human health and obesity this allows an additional level of association to be developed between certain baseline characteristics of participants (in this instance breakfast consumption/skipping) and development of conditions. However, it should be noted that studies utilising this design still do not provide causal evidence relating the variable of interest with the outcomes assessed. Because of their longer term nature, these studies tend to be less prevalent in the literature but there have been some examinations linking breakfast consumption with a variety of disease and weight change outcomes.

Body Mass Change with Breakfast Consumption

Breakfast consumption has been clearly associated with excess weight in the cross-sectional associations already presented. Whether breakfast consumption is associated with weight change in adults is less comprehensively examined. However, there is some consistency in the findings garnered. In 20064 male health professionals who were between 40-75 y at baseline, a 10 year follow-up period demonstrated reduced risk of 5% BMI gain in those that reported usually eating breakfast at baseline (van der Heijden et al., 2007). The odds ratio for 5% BMI gain in the individuals classified as breakfast consumers was 0.87 (95 % CI, 0.82-0.93) when accounting for other relevant health mediators (e.g smoking status, physical activity quintile, alcohol intake). Interestingly, this reduction in risk was stronger in individuals with a BMI < 25 at baseline (0.78, 95% CI, 0.70-0.87), relative to those individuals that were >25 at baseline (0.92, 95% CI, 0.85-1.00). However, in breakfast "non-consumers" a greater proportion of participants reported dietary intake before breakfast (3.9 *vs* 0.4 %) and between breakfast and lunch (21.9 *vs* 7.4 %) than breakfast consumers. Therefore this suggests that ~20-25% of the "non-consumer" cohort did not undertake an unbroken fast prior to lunch, limiting their discrepancy from the consuming group somewhat. Despite this general consideration, this work provides some evidence that the

potentially protective association of breakfast consumption against weight loss may be mediated by an individual's weight status.

Goto and colleagues (2010) have reported in a substantially younger population of 4634 Japanese University students (21.5 ± 1.9 y) with a baseline BMI of >22, that 1 year BMI gain of 5% was more prevalent in those reporting skipping breakfast twice a week or more (OR 1.34, 95 % CI, 1.12-1.61). This demonstrates that more rapid weight gain (i.e the same BMI gain endpoint but in 1 rather than 10 years) than assessed in the work of van der Heijden and colleagues (2007) is also apparent in those that report skipping breakfast more frequently. In addition to these findings, Nooyens et al. (2005) reported that the change in breakfast consumption frequency over a 5 year follow up period was associated with increased weight in 288 Dutch 50-65 year olds. However, this relationship which originally related an increase in each unit of breakfast consumption frequency to greater weight gain of 0.07 kg per year (p = 0.03), was moderated to 0.04 kg per year and was no longer significant (p = 0.2) when accounting for other covariates. Therefore, although this study does not provide evidence that increasing breakfast frequency is associated with weight gain when appropriate adjustments are made, it also does not provide support for increasing breakfast frequency as associated with reducing weight gain. However, it is important to consider that this study investigated the effect of *changing* breakfast habits upon weight outcomes, and yet in the 4 subgroups studied the greatest potential range of change in breakfast frequency (95% CI) was -0.47 occasions per week in a group whose mean change in frequency was -0.16 and 0.89 in a group whose mean change was 0.44 occasions per week, indicating that the magnitude of change in this variable over the course of the follow up period was in fact very small, somewhat limiting the application of this analysis.

In a more nuanced analysis of the relationship between breakfast consumption and weight gain, Purslow and colleagues (2008) examined the relationship between energy intake at breakfast and ~4 year weight gain amongst 6,764 men and women aged 40-75. The experimenters split individuals into quintiles based upon proportion of total energy intake (as assessed by 7 day food diary) consumed at breakfast. They report that as the proportion of daily energy intake from breakfast decreased, weight gain increased (p < 0.001). Those in the lowest quintile for EI at breakfast gained (±

SEM) 1.23 ± 0.12 kg compared with 0.79 ± 0.11 kg in those in the highest quintile. As observed in the majority of cross-sectional studies discussed earlier, these authors also report that as energy intake from breakfast increased, so did total energy intake ($p < 0.001$). Therefore, individuals reporting greater energy intake were less likely to gain weight, suggesting a potential role for energy expenditure in moderating the relationship between breakfast consumption and weight change.

Taking a similar approach in attempting to move beyond straightforward categorical (i.e yes/no) definitions of breakfast consumption, Odegaard and colleagues (2013) have reported that over an 18 year follow-up period, that those individuals reporting breakfast consumption both daily and 4-6 days per week had lower weight gain than those who ate breakfast on 3 or less days a week. Specifically, daily consumers gained 1.9 kg less over the period than the infrequent breakfast consumers.

Breakfast Consumption and Disease Risk

There is a recently emerging body of evidence from prospective studies that is beginning to link breakfast consumption and disease risk in individuals. Cahill and colleagues (2013) have examined the incident coronary heart disease risk in the Health Professionals Follow Up Study. Men aged 45-82 (n=26902) who were free of cardiovascular disease and cancer at baseline, were asked about eating habits in 1992 and followed up for 16 years. The research group examined the frequency of non-fatal myocardial infarctions and deaths from coronary heart disease and found that those reporting not eating in the morning had a 33% greater age adjusted risk of CHD incident (95% CI, 1.13-1.57). When adjusting comprehensively for diet, demographic and physical activity factors this risk was only slightly reduced (1.27, 95 % CI, 1.06-1.53). However, when also adjusting for BMI and other health conditions (presence of diabetes, hypercholesterolemia, hypertension) this risk was reduced again and to a greater extent to 1.18 (95 % CI, 0.98-1.43, $p = 0.08$).

Utilising the same dataset, Mekary and colleagues (2012) have established a similar relationship between breakfast skipping and cases of diabetes. Individuals who reported skipping breakfast had a 27% greater risk of diabetes (95% CI, 1.13, 1.43) when accounting for numerous other potential risk factors, this risk was reduced slightly when BMI was also included (1.21, 1.07-1.35). Interestingly, these

relationships were not different whether comparing people who did not eat anything prior to lunch or those who simply reported not eating breakfast (but did report eating at another occasion prior to lunch). The same investigators have also examined this relationship in women in a partner study (Mekary et al., 2013). In this investigation, daily breakfast consumption was compared with irregular consumption (6 days per week or less) and followed up after 6 years. These results were remarkably similar, with the risk factor adjusted OR 1.28 (1.14-1.44) in those not consuming daily, which was attenuated to 1.20 (1.07-1.35) when BMI was also included. Unfortunately, despite the original questionnaire including frequency of breakfast consumption questions (i.e how many days per week breakfast was consumed), the authors chose to dichotomise this variable, so further information is not available relating to the frequency of breakfast consumption and relative risk in this cohort.

In an analysis that has utilised a more detailed description of breakfast consumption frequency, Odegaard and colleagues (2013) have demonstrated a stepwise reduction in metabolic risk with increased breakfast consumption frequency. The authors report that when comparing infrequent (0-3 days per week), frequent (4-6 days per week) and daily consumers, that in all cases, daily consumption was associated with the greatest reduction in risk of incidence of abdominal obesity, obesity, metabolic syndrome, hypertension and type 2 diabetes. This evidence suggests that greatest protection from development of metabolic risk is conferred with the highest frequency of breakfast consumption, with a dose-response relationship evident.

These prospective studies examined provide consistent evidence that omission of breakfast is longitudinally associated with increased risk of weight gain over time and that potentially increased frequency of breakfast consumption is beneficial in preventing weight gain. Several studies have also established that breakfast omission is associated with a greater risk of the development of metabolic risk and disease states, even when other relevant variables are accounted for. Despite this, these studies do not provide evidence to support potential mechanisms to explain the associations observed.

1.12 Randomised Controlled Trials

Despite the large volume of associative studies examining breakfast consumption, there are relatively few studies that have attempted to establish the causal role of breakfast consumption/omission upon components of energy balance. Whilst there have been several studies that have examined responses to various different compositions of breakfast, very few have examined the variable of particular interest in this thesis; the morning fast. The studies that have examined the relative presence versus absence of breakfast *per se* can be split into two main categories: acute studies (e.g laboratory based investigations conducted within a day) and chronic interventions (e.g adherence to a prescribed breakfast or no breakfast routine, typically over multiple days or weeks).

1.12.1 Acute Studies

Acute breakfast studies are generally used to provide detailed information relating to appetite regulation and energy intake. Whilst these studies can provide some information relating to minor parts of energy expenditure (i.e DIT), the restrictive nature of the environment means acute studies do not provide a comprehensive picture of energy expenditure, due to restrictions upon physical activity.

One of the potential mechanisms that omission of breakfast may act through to affect energy balance is alteration of energy intake. If there is a causal component to the association of breakfast skipping and obesity, this may be due to increased energy intake after fasting. Therefore, despite the absence of energy intake through breakfast, it could be that breakfast skippers overcompensate through other feedings later in the day, resulting in greater overall (absolute) energy intake with breakfast skipping. However, the majority of cross-sectional evidence presented previously does not support this notion (Cho et al., 2003; Deshmukh-Taskar et al., 2010a; Nicklas et al., 1998; Schusdziarra et al., 2011; Purslow et al., 2008).

In two related studies, Levitsky and Pacanowski (2013) investigated whether breakfast skipping results in compensatory increases in energy intake at other feeding occasions. In their first experiment, the authors provided either a high carbohydrate or

fibre breakfast of ~335 kcal and compared these breakfast conditions against a breakfast omission condition in 24 predominantly female university students. They report no difference (all conditions within 50 kcal) in energy intake at an *ad libitum* lunch, thus a significantly greater absolute intake in the breakfast conditions than the morning fasting condition. The authors speculated that this may be due to the relatively small breakfast and/or that energetic compensation may occur throughout the day, leading them to conduct a further investigation. In a similar population, the authors then provided a variety of food items in a buffet style breakfast or asked participants to skip breakfast. Subsequently, a buffet style lunch was eaten within the research unit, with snacks and dinners that could be eaten outside the laboratory. In this investigation they found that in the morning fasting trial, lunch intake was approximately 170 kcal greater ($p = 0.04$) than in the breakfast condition. However, no other eating occasions were significantly affected by morning breakfast or skipping, such that when the energy intake from breakfast (624 kcal) was included, those that had fasted in the morning had consumed ~450 kcal less ($p < 0.01$) over the course of the day. Consistent with the lack of energetic compensation throughout the rest of the day, hunger was assessed as significantly greater in the morning fasting trial during the mid-morning and prior to lunch but not different from the breakfast trial from the afternoon onwards.

Two further investigations in young, lean men have contrasted an extended morning fast with a breakfast consumption condition, also including metabolic and hormonal measures as well as investigating energy intake compensation to extended morning fasting (Astbury et al., 2011; Gonzalez et al., 2013). These two investigations provide contrasting results, with one investigation reporting greater energy intake at an *ad libitum* lunch sufficient to compensate for omitted breakfast energy intake following extended fasting (Astbury et al., 2011) and the other no difference in intake at lunch (Gonzalez et al., 2013). These discrepant results may be partly due to differences in the morning feedings in the two studies, with the former investigation providing less energy at breakfast (~270 kcal *vs* 444 kcal). Additionally, a crucial point is that in both the breakfast and no breakfast condition, participants consumed a mid-morning preload (i.e a snack), such that in the no breakfast condition participants had not undertaken an unbroken fast prior to consuming lunch. In the work of Gonzalez and colleagues (2013) the preload was greater (358 kcal *vs* 250 kcal) than in the study of Astbury and colleagues (2011). These two differences in the size of the feedings

provided may partly explain the discrepant findings, as the similar total energy intake between those consuming and skipping breakfast in the work of Astbury et al (2011) may be because those not eating breakfast had "missed" less energy intake at breakfast compared with Gonzalez et al (2013) and had also been provided less energy in the form of preload prior to eating lunch.

Both investigations show that appetite was increased in those not consuming breakfast throughout the morning leading to lunch. Breakfast omission resulted in a tendency for (Gonzalez et al., 2013) and greater (Astbury et al., 2011) glucose and insulin responses to the mid-morning preload, with both investigations reporting greater free fatty acid concentrations during the morning in those omitting breakfast. GLP-1 was not different following the preload in the work of Gonzalez et al. (2013), but was lower 15 minutes after the preload (as was PYY at 15 and 30 minutes) in those who had skipped breakfast in the work of Astbury et al. (2011). Astbury and colleagues (2011) also monitored appetite hormones and metabolic variables after lunch, reporting no difference between any of the measured outcomes in the breakfast and no breakfast trials.

While both of these studies provide relevant and useful information analogous to the mechanisms of appetite regulation when those skipping breakfast choose to consume a mid-morning snack, the responses to unbroken fasting prior to lunch have not been examined in a laboratory setting.

1.12.2 Intervention Studies

Whilst the acute interventions above can provide valuable mechanistic information relating to short term responses to a single exposure of either breakfast or morning fasting, intervention studies allow examination of responses to chronic exposure to either morning fasting or breakfast outside the laboratory environment.
The studies that have examined a breakfast omission intervention in a randomised controlled trial are summarised in Table 1.3. As can be seen, there are few randomised controlled trials that have investigated an extended morning fast.

Table 1.3: Intervention studies that have examined a breakfast omission condition

Author	Participants	Design	Intervention	Restrictions	Key Outcomes
(Dhurandhar et al., 2014a)	N=283 Both genders 20-65 y BMI 25-40 "Interested" in weight loss Mixture of skippers and consumers	16 weeks, multicentre trial 3 parallel groups Control, Breakfast, No Breakfast.	**Control**-Nutrition Pamphlet **Breakfast**-Nutrition pamphlet + instruction to eat breakfast prior to 10am **No Breakfast**-Nutrition pamphlet + instruction to not consume anything other than water/zero calorie beverages until 11:00.	None	No difference in weight loss between treatments. No difference between individuals classified as skippers (breakfast <4 per week) and consumers (breakfast ≥4 per week) at baseline on weight loss.
(Farshchi et al., 2005b)	N=10 women 19-38 y BMI 23.2 ± 1.4 All regular breakfast consumers	6 weeks, crossover design. 2 weeks on each intervention, 2 week washout period in middle.	**Breakfast**-45 g Bran flakes with 200 ml milk between 07:00 and 08:00, chocolate covered cookie between 10:30 and 11:00 **No Breakfast**-Chocolate covered cookie between 10:30 and 11:00 and cereal with milk between 12:00 and 12:30	Asked to consumed 2 more meals and 2 snacks of usual content at predetermined times throughout day. Dinner to be consumed between 18:00 and 18:30	No effect on weight. Reported energy intake lower in breakfast condition. Increased total and LDL cholesterol following no breakfast. AUC of insulin to test meal lower after breakfast than no breakfast.
(Halsey et al., 2012)	N=49 Both genders 22.6 ± 3.9 y BMI not specified Mixture of skippers and consumers	2 weeks, crossover design 1 week on each intervention, no washout period	**Breakfast**-*Ad libitum* intake of high carbohydrate foods between 08:00 and 09:00, tea/coffee permitted in morning, no other foods allowed until 12:00 **No Breakfast**-No food intake in the morning, tea and coffee permitted throughout morning.	Participants had to report to feeding lab each morning between 08:00 and 09:00. Participants returned to lab between 17:00 and 18:00 to return monitors. No diet restrictions.	No difference in energy intake between conditions. No difference in mean pedometer scores or heart rate.

Table 1.3 continued: Intervention studies that have examined a breakfast omission condition

Author	Participants	Design	Intervention	Restrictions	Key Outcomes
(Reeves et al., 2014)	N=37 Both genders Lean (30 ± 8 y) and overweight/ obese groups (36 ± 16 y) Mixture of skippers/consumers	1 week interventions, crossover design ≥1 week washout period.	**Breakfast**-Asked to eat breakfast by 10am. **No Breakfast**-Refrain from eating until midday.	None	Greater energy intake with breakfast consumption. No effect of habits or BMI upon energy intake.
(Schlundt et al., 1992)	N=52 women 18-55y BMI 30.6 ± 0.5 Mixture of skippers/consumers	12 weeks, parallel groups, associated behavioural intervention	**Breakfast**-400 kcal breakfast as part of menu **No Breakfast-**No breakfast prescribed	1200 kcal menus provided to participants, specifying meal energy intakes.	Weight loss in both groups. Habit x intervention interaction (p = 0.06) for weight loss, those who changed from their normal breakfast habit during intervention lost more weight.
(Stote et al., 2007)	N=15 both genders 40-50 y BMI 18-25	Randomised crossover design, 2 x 8 week interventions, 11 week washout	**Breakfast-**Not specified **No Breakfast**-Nothing permitted until all energy intake consumed within one meal between 17:00 and 21:00	Participants not permitted to consume foods other than those provided by or consumed in the research facility. Feeding frequency was fixed at either 3 meals/d or 1 meal a day. Energy intake was designed to maintain energy balance.	Body and fat mass loss in 1 meal/d group. Increases in all measures of cholesterol in 1 meal/d regimen. No difference in physical activity.

Effect on Body Mass

The effect of extended morning fasting upon body mass is not well established in randomised controlled trials. Several of the studies conducted have been of a relatively short term nature (≤ 2 weeks), such that weight change was not a measure obtained (Halsey et al., 2012; Reeves et al., 2014) or was not expected to change (Farshchi et al., 2005b). In two other studies, weight loss was either part of the intervention as a calorie restricted diet was prescribed (Schlundt et al., 1992) or weight loss was attempted to be avoided as participants were provided with foods to attempt to maintain energy balance (Stote et al., 2007). Despite these energy balance prescriptions, some differences in body composition changes were apparent between morning fasting and breakfast consumption. In the work of Schlundt and colleagues (1992) there were no differences in weight loss between the breakfast and fasting interventions. However, there was a tendency (p = 0.06) for greater weight loss in those individuals that changed from their habitual behaviour during the intervention (i.e a habitual breakfast skipper asked to consume breakfast during the intervention and vice versa). Stote and colleagues (2007) report greater total mass and fat loss in those consuming only 1 meal per day relative to a 3 meal a day regimen. This difference may be partly attributable to the slight reduction in provided energy intake during the 1 meal regimen of 65 kcal daily. Additionally, it is important to acknowledge that although this intervention did prescribe an extended morning fast, it also included omission of lunch as well so is not an equivalent comparison to a morning fasting intervention.

Therefore, only one randomised controlled trial remains that examined body mass change with breakfast omission. The study of Dhurandhar et al (2014) implemented a pragmatic intervention involving a recommendation to either consume breakfast daily by 10:00 or to not consume anything prior to 11:00 for 16 weeks in overweight/obese men and women. They report no difference in weight change between those asked to consume or skip breakfast. Additionally, despite using a similar definition of habitual breakfast consumption (self-reported frequency ≥ 4 days per week) as Schlundt and colleagues (1992), they report no effect of pre-randomisation consumption habits upon weight change. Considering the much greater sample size (283 *vs* 52) in the more recent work, the authors have suggested that the proposed effect of prior breakfast habit upon weight change in an intervention may

have been a type 1 error or specific to the intensive nature of the weight loss programme delivered in the Schlundt study.

Effect on Metabolic Outcomes

Only two studies have examined the metabolic outcomes arising from an extended morning fasting intervention (Farshchi et al., 2005b; Stote et al., 2007). The work of Stote and colleagues (2007) established that individuals only consuming one large meal in the evening for 8 weeks had increased concentrations of total, HDL and LDL cholesterol concentrations at follow-up. In a less severe and more realistic extended morning fasting intervention, Farshchi and associates (2005) report increased total and LDL cholesterol in participants who were asked to delay their first feeding occasion until 10:30 each morning. In this study, the authors also report no difference between fasting measures of insulin sensitivity, or blood glucose and insulin profiles to a mixed macronutrient drink. However, a treatment x visit interaction was apparent ($p = 0.001$) for insulin iAUC, with a reduction in insulin response following breakfast consumption and an increase after breakfast skipping for 2 weeks. This finding was suggested as potentially a reason for the increased cholesterol concentrations observed following the no breakfast condition, due to greater insulin stimulation of hydroxyl methyl glutaryl-Co-A reductase (one of the rate limiting enzymes in cholesterol synthesis).

Effects on Components of Energy Balance

Of the randomised controlled trials identified, none of the investigations have quantified all elements of energy balance. All of the investigations with the exception of Dhurandhar and colleagues (2014) have attempted to measure energy intake. However, in two of these studies, energy intake was prescribed and therefore is not a reflection of volitional eating habits, but the successful implementation of the intervention (Schlundt et al., 1992; Stote et al., 2007). Of the three studies that remain and did not explicitly define an energy intake target, results obtained are equivocal, with increased (Farshchi et al., 2005b), similar (Halsey et al., 2012) and reduced (Reeves et al., 2014) total daily energy intake in those that did not consume breakfast.

These varying reported energy intakes may have arisen from differences in the nature of the morning fasting and breakfast interventions. In the work of Farshchi and colleagues (2005), feeding frequency was strictly prescribed throughout the day, with 4 time windows for meal and snack intakes following consumption of the same food items in the morning in the two conditions (but delayed by 2-3 hours in the no breakfast condition). In a slightly less prescriptive model, Halsey and colleagues (2012) detected no difference between the morning fasting (no food consumption until 12:00) and breakfast interventions. However, in this investigation, participants had to report to the laboratory in the morning to consume a prescribed high carbohydrate breakfast *ad libitum*. In the only study that has not prescribed either breakfast composition, or any other aspect of feeding behaviours, energy intake was found to be reduced when individuals were asked to not consume foods prior to 12:00 (Reeves et al., 2014). It therefore appears that the reported energy intake of participants may be partly dependent upon the level of dietary prescription imposed, and that individuals freely choosing all aspects of feeding may consume less energy daily when fasting during the morning.

Only two studies have measured changes in RMR in response to a morning feeding intervention (Farshchi et al., 2005b; Schlundt et al., 1992). Of these, the work of Schlundt and colleagues (1992) demonstrated that the weight loss induced by caloric restriction in both the breakfast consumption and morning fasting trials resulted in similar reductions in RMR, with no differences between the two feeding regimens. In accord with these findings, in the two week intervention of Farshchi and associates (2005), there was no difference in RMR (or weight/body composition) following either the breakfast consumption or skipping regimens. This limited evidence suggests that consistently extending a morning fast does not differently affect RMR, beyond the associated responses that would be predicted, based upon the relative stability or fluctuations of body composition induced by interventions.

The two least well understood components of energy expenditure are DIT and physical activity energy expenditure. Diet induced thermogenesis after an intervention has only been examined by Farshchi et al (2005), with the authors reporting no effect on the thermic effect of a mixed macronutrient test drink after either of the morning feeding interventions undertaken. Whilst this is a component of energy expenditure

that warrants further investigation, DIT contributes a small proportion towards energy expenditure. Undoubtedly, the most quantitatively significant and malleable component of energy expenditure is physical activity thermogenesis, although this has received surprisingly little attention.

Only two of the studies identified have examined the impact of their interventions upon free-living physical activity. Stote and colleagues (2007) report no difference in physical activity measured by accelerometry between individuals consuming either 1 or 3 meals per day. It should be noted that participants "were encouraged to maintain their normal exercise throughout the study", so it is entirely plausible that any effects of the feeding interventions may be precluded by the instruction to continue normal habits. The other intervention that has attempted to measure physical activity is the work of Halsey and colleagues (2012). This study utilised a combination of pedometers and HR monitors to attempt to quantify differences in physical activity. However, these devices were used in University students on campus, that had to report to the laboratory to consume breakfast or tea/coffee in the morning (where they were provided with the monitors) and then returned the monitors between 17:00 and 18:00 each day. Therefore, these records provide very incomplete assessments of activity as they were only worn for approximately 8 hours a day, and during a time where the majority of their activities would be expected to be dictated by their University schedules. It is therefore not surprising that apart from an increase in HR during the morning (presumably due to DIT) there were no other observed differences in the measures for the breakfast consumption and skipping conditions.

1.13 Aim of Thesis

This review of literature has examined the evidence relating to omission of breakfast upon energy balance and health. Despite entrenched beliefs that breakfast consumption has several positive effects for health, the scientific evidence for this contention is not convincing. A wealth of cross sectional and prospective literature supports that omission of breakfast is associated with an increased risk of adiposity but is also associated with a number of other risk factors for obesity and poorer health (e.g lower physical activity, greater alcohol consumption etc.). This cross sectional evidence cannot be used to establish the existence or direction of a causal relationship between breakfast consumption and health. Evidence from randomised controlled trials is lacking, with no short term studies examining the hormonal and metabolic responses to unbroken fasting prior to *ad libitum* lunch intake. Free-living interventions have generally been of relatively short durations (i.e 1-2 weeks), and not measured all components of energy balance or have placed several restrictions upon participants. Therefore, the aim of this thesis is to examine the effect of morning fasting in both laboratory and free-living studies. A variety of measurement techniques will be employed to investigate the effects of morning fasting upon components of energy balance and human health in both lean and obese individuals.

Chapter 2: General Methods

2.1 Participant Eligibility Criteria

Inclusion Criteria

- Aged 21-60
- Body Mass Index 20-25 $kg \cdot m^{-2}$ or >30 $kg \cdot m^{-2}$
- Able and willing to safely comply with all study procedures
- Able to provide written informed consent for participation
- Females must maintain a record of regular menstrual cycle phase or contraceptive use
- No anticipated changes in diet and/or physical activity habits during the study period (e.g. pre-planned holidays, diets/exercise plan, *etc*.)

Exclusion Criteria

- Any reported condition or behaviour deemed either to pose undue personal risk to the participant or introduce bias into the experiment
- Any reported use of substances which may pose undue personal risk to participants or introduce bias into the experiment
- Any individual whose habitual lifestyle does not conform to a standard sleep-wake cycle (e.g. shift workers)
- Simultaneous or recent (i.e. last 3 months) participation in another clinical trial or blood donation, to allow full recovery of blood volume (Pottgiesser et al., 2008)
- Any reported recent (i.e. last 6 months) shift (>2%) in body mass, this criteria was based upon a more stringent interpretation of the classification of 3% variability set out by Stevens *et al.* (2006) as weight maintenance.
- Any reported tendency towards keloid scarring
- Any reported bleeding disorder
- Females known to be pregnant or planning to become so over the course of the study

- Females with oral or implanted contraceptives fitted within 6 months of participation, due to reported effects upon carbohydrate metabolism (Biswas et al., 2001).
- Females who are breastfeeding

2.2 Overall Experimental Design

The general design of the study involved three experimental phases attempting to address three main research questions.

- Phase 1) Using a randomised, counterbalanced crossover design to establish the acute effects of morning fasting and breakfast consumption on energy intake, appetite and metabolic responses in a laboratory based protocol.
- Phase 2) Using a randomised, parallel groups design to establish the effects during free-living of chronic adherence to a daily morning fasting or breakfast consumption intervention for 6 weeks on all aspects of energy balance and markers of metabolic control and cardiovascular disease risk.
- Phase 3) Establish if the chronic intervention in Phase 2 causes adaptation of the acute responses measured in a repetition of the breakfast consumption trial from Phase 1 after completion of Phase 2.

These three phases ran consecutively in the same participants (both lean and obese), with the progression through the project displayed in Figure 2.1.

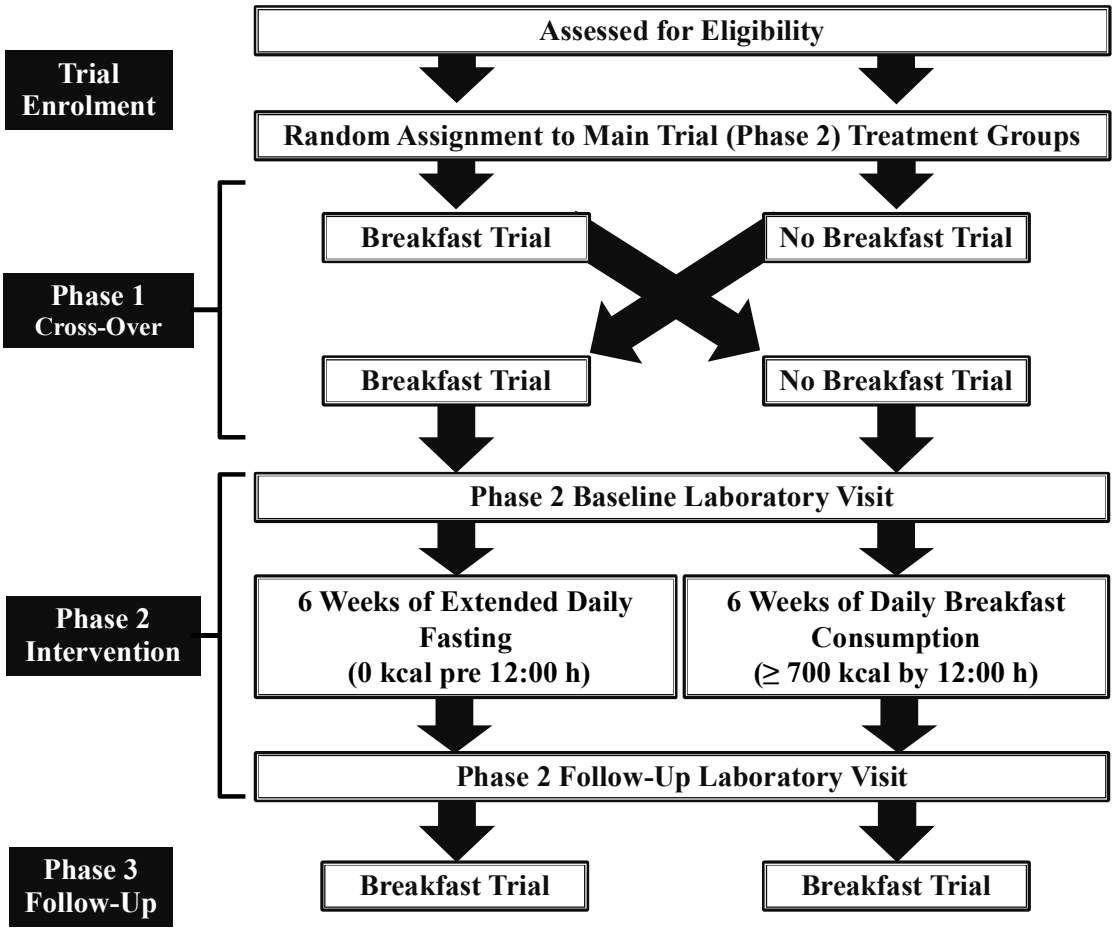

Figure 2.1: Overall experimental design showing the progression of participants through the phases of the project.

2.3 Allocation to Experimental Groups

The aim of the project was to test a total cohort of 60-70 men and women in the main part of the experiment, half of whom were broadly classified as normal weight (BMI ~20-25 $kg \cdot m^{-2}$), and the other half obese (BMI ≥30 $kg \cdot m^{-2}$). The broad classification according to BMI was intended to generate two separate and diverse overall populations for subsequent more accurate and gender specific stratification based upon DEXA-derived fat-mass index (♂ FMI ≤7.5 $kg \cdot m^{-2}$<; ♀ FMI ≤11 $kg \cdot m^{-2}$<), thus controlling for differences in lean tissue (Kelly et al., 2009) allowing separate analyses according to obesity status. Furthermore, baseline breakfast habits were assessed prior to intervention, and included in our randomisation scheme to broadly distribute habitual consumers and those that omit breakfast within the experimental groups, with frequent breakfast consumption defined as the ingestion of ≥50 kcal within two hours of waking on most days of the week. Both the above factors were included in a stratified randomisation scheme, with generation of 4 separate block randomisation schedules. These randomisation schedules were produced by the principal investigator using a computer-based random number generator.

2.4 Sample Size Estimation

The number of research participants to be recruited was estimated based upon a worthwhile effect dictated by the smallest shift in energy balance necessary to induce chronic weight loss. In a population not dissimilar to the participants to be used (Verboeket-van de Venne et al., 1993), daily energy expenditure measured using doubly labelled water from which a mean of ~2870 kcal·d^{-1} was established with a standard deviation in the region of ~480 kcal·d^{-1}. The daily breakfast to be consumed in the free-living part of this thesis will provide in excess of ~700 kcal·d^{-1} with pilot work using this breakfast indicating that this is likely to stimulate metabolism in the lead up to lunch by ~35 kcal·d^{-1} and reduce energy intake at lunch by ~35 kcal·d^{-1}, relative to continued fasting. Therefore, for breakfast consumption to exert a worthwhile effect on net energy balance (sufficient to compensate for the energy it provides directly), physical activity energy expenditure would need to increase and/or subsequent energy intake to decrease by a further 640 kcal·d^{-1}. Using the above figures, a worthwhile increase in energy expenditure would require ~14 participants in each treatment group to confer a 90% probability of detecting such an effect statistically using a two-tailed *t*-test with an alpha level of 0.05.

2.5 Standardisation Prior to Laboratory Visits

Participants were assigned (but did not commence; Figure 2.1) their free-living intervention prior to attending the laboratory for their first laboratory trial. Participants were asked to fast overnight (≥10 h) prior to arrival at 08:00 ± 1 h, with subsequent trials commencing within an hour of the first visit and the timeframe stated above. In the 48 h prior to their first lab visit for each phase of the trial, participants kept a weighed food diary, which they subsequently replicated prior to corresponding visits to the laboratory. Participants were permitted to keep two food diaries, one for feeding trials in phases 1 and 3, and another for the pre- and post-intervention laboratory visits in phase 2. During the two mornings prior to all laboratory visits, participants were asked to follow their assigned free-living intervention dietary pattern (i.e ≥700 kcal by 11:00 or fasting until 12:00). In the 24 h before a lab visit, individuals were asked to refrain from caffeine and alcohol intake and avoid strenuous physical activity. Upon waking on the day of laboratory testing participants consumed 568 ml of plain water to promote adequate hydration. Between laboratory visits participants either continued their normal lifestyles or undertook their free-living intervention as appropriate (described in further detail in relevant chapters).

2.6 Laboratory Methods

2.6.1 Blood Sampling

All bloods were obtained via cannula inserted into the antecubital fossa of participants, with the cannula kept patent through regular flushing with 0.9% Sodium Chloride infusion (B.Braun, UK). Samples were drawn from a 3-way tap, with the first 5 mL of blood drawn discarded to avoid contamination with saline.

Plasma Samples

Blood was dispensed into tubes coated in the anti-coagulant ethylenediaminetetraacetic acid (EDTA) (Sarstedt Ltd, UK). They were then immediately centrifuged at 3466 x g rpm for 10 minutes at 4 ˚C (Biofuge Primo R, Heraeus, Germany), with the plasma fraction removed and cooled using dry ice until subsequent storage at -80 ˚C.

Serum Samples

Blood was dispensed into a plain blood tube with no anti-coagulant treatment (Sarstedt Ltd, UK) and left at room temperature to clot for 45 minutes. They were then centrifuged at 3466 x g for 10 minutes at 4 ˚C (Biofuge Primo R, Heraeus, Germany). The serum fraction was then removed and cooled using dry ice until storage at -80 ˚C.

Samples for Analysis of Acylated Ghrelin

One mL of whole blood was dispensed into a tube coated in EDTA (Sarstedt Ltd, UK) which had 50 µL of a p-hydroxymercuribenzoic acid solution (prepared as a 100 mM concentrate solution in potassium phosphate buffer containing 1.2% 10 N NaOH) added to prevent the degradation of acylated ghrelin by proteases (Chandarana et al., 2009). Samples were then centrifuged at 3466 x g for 10 minutes at 4 ˚C. Five hundred µL of the supernatant was then transferred to an untreated blood tube to which 10 µL 1 N hydrochloric acid had been added. Samples were centrifuged again as described above, with the supernatants removed, cooled using dry ice and then stored at -80 ˚C.

Arterialisation of Venous Bloods

In obese participants during collection of blood samples for measurement of glucose and insulin for assessment of insulin sensitivity in Chapter 7, a "heated hand" technique for arterialisation of venous blood was used (Gallen and Macdonald, 1990). This technique was employed to enable measurement of blood glucose more reflective of arterial levels prior to metabolism by the tissues of the forearm.

Prior to any measurements being obtained the hand was warmed for 15 minutes, which has been demonstrated by Kurpad et al (1994) to cause sufficient arterialisation, as demonstrated by increased PO_2 from 67 % to 96 % following 10 minutes of heating. The chosen method utilises static air at 55 ˚C to heat the hand, which has previously been shown to minimally affect core temperature (Gallen and Macdonald, 1990), and cause less hemodynamic disturbance than use of direct heating using a warming blanket (Blaak et al., 1992). As suggested by Frayn and MacDonald (1992), to reduce the potential muscle mass for metabolism, cannulation was performed retrograde in the dorsal veins of the heated hand.

Quantitatively, in the context of hyperglycaemia induced during a clamp procedure, McGuire and colleagues (1976) have indicated that in a forearm vein the A-V difference in glucose was approximately 43 % 2 minutes into glucose infusion, and reduced to around 15 % by the end of the 70 minute clamp procedure. In a heated hand condition this difference was around 11 % at 2 minutes and was reduced to the basal difference by 50 minutes. While this response may be exaggerated relative to the oral glucose tolerance test being conducted in the present work; it would seem to indicate that metabolism of the tissues in the forearm is a potentially significant moderator of measured blood glucose, particularly within a hyperinsulinaemic environment as would be expected (at least transiently) during a glucose challenge.

This methodology only became available for use in our laboratory following completion of the majority of the testing of lean participants as presented in Chapter 4. While this must be taken into consideration when attempting to directly compare between participants in the lean and obese groups, for the main comparisons of pre-post intervention insulin sensitivity within groups, there is no evidence to suggest that

the pattern of results obtained using venous blood would be different in response to the intervention.

2.6.2 Analysis of Blood Samples

Analysis of blood samples for concentrations of triglycerides, non-esterified fatty acids (NEFA), glucose, C-Reactive Protein (CRP), urea, total cholesterol and high density lipoprotein (HDL) cholesterol were conducted on a Daytona (Randox Laboratories, Crumlin, NI) automated analyser according to manufacturer instructions using commercially available immunoassays (Randox Laboratories, Crumlin, NI). Low density lipoprotein (LDL) cholesterol was estimated using the Friedewald equation (Friedewald et al., 1972). This equation calculates LDL cholesterol as the following:

LDL Cholesterol = Total Cholesterol – HDL Cholesterol – (Triglycerides / 2.2)

Total and acylated ghrelin (Bertin Pharma, Montigny le Bretonneux, France), PYY and total GLP-1 (Merck Millipore, Billerica, USA), adiponectin and leptin (R&D Systems Inc., Abingdon, UK) and insulin (Mercodia AB, Uppsala, Sweden) analyses were conducted using commercially available enzyme-linked immunosorbent assays (ELISA) according to manufacturer instructions. For all blood analyses, all samples for each participant (i.e each laboratory visit for the same person) were completed on the same ELISA plate or analyser run. Where samples were undetectable by the analysis method employed, they were included as the lowest detectable concentration for the purposes of statistical analysis. Further details of the biochemical analyses conducted are included in Table 2.1.

Table 2.1: Intra-assay and inter-assay coefficient of variation of assays employed*

Parameter	Intra-Assay (CV)	Inter-Assay (CV)
Total Cholesterol	< 4 %	< 2 %
HDL Cholesterol	< 4 %	< 3 %
Triglycerides	< 4 %	< 4 %
NEFA	< 5 %	< 5 %
CRP	< 3 %	< 5 %
Glucose	< 5 %	< 6 %
Urea	< 5 %	< 3 %
Total Ghrelin	4.0 %	7.8 %
Acylated Ghrelin	4.2 %	11.3 %
PYY	4.3 %	11.1 %
GLP-1	4.8 %	27.0 %
Adiponectin	4.0 %	6.3 %
Leptin	3.4 %	6.4 %
Insulin	4.7 %	12.5 %

*Where intra- and inter-assay CV is displayed as "less than" the CV is established across multiple quality controls, therefore the greatest of these is listed.

2.6.3 Urine Collection

Participants' urine was collected in a vessel containing 5 mL of 10 % thymol isopropanol which acted as a preservative. For a given measurement period the collected urine was mixed thoroughly, with a 1 mL aliquot obtained and kept at -80 ˚C prior to analysis. Analyses of urinary urea were conducted using a commercially available immunoassay on a Daytona automated analyser according to manufacturer instructions (Randox Laboratories, Crumlin, NI).

2.6.4 Expired Gas Analysis

All samples of expired air were collected for a 5 minute duration. A minute prior to commencement of sampling, participants were provided with a nose clip and the respiratory valve in order to clear any atmospheric air from the apparatus. The respiratory valve was connected to a 200 L Douglas Bag (Hans Rudolph, MO, USA) via falconia tubing (Baxter, Woodhouse and Taylor, UK). The obtained samples were then run through tubing containing anhydrous calcium sulphate (Drierite, OH, USA) to remove water content from the samples. Relative proportions of O_2 and CO_2 were then measured in a known volume of the sample using paramagnetic and infra-red analysers, respectively (Servomex 1440, UK). This analyser was calibrated on the morning of each testing day using two gases of known composition of ~0 % and 16 % O_2 and ~0 % and 5 % CO_2, respectively (British Oxygen Company, UK). The volume of expired air (V_E) was established using a dry gas meter (Harvard Apparatus, UK), with the temperature of the expired gases measured using a CheckTemp1C (Hanna Instruments, RI, USA) during evacuation from the Douglas bags.

Where possible, atmospheric conditions within the laboratory were obtained for each individual gas collection due to the potential effects of differences in atmosphere within confined environments (Betts and Thompson, 2012). This involved measurement of laboratory temperature using a Testo 625 thermohygrometer (Testo, UK) and measurement of the composition of inspired gases (within ~1 metre of the participant during expiration into the Douglas Bag) as described above. Where this was not feasible, established laboratory norms for O_2 and CO_2 were used instead.

2.6.5 Calculation of Energy Expenditure

Expired air samples were converted to the standard temperature (0 ˚C) and pressure (760 mmHg) for a dry gas. The Haldane Transformation was used to determine inspired gas volumes (V_I), such that rates of both oxygen utilisation ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) could be calculated (Frayn, 1983). These rates were then used to calculate energy expenditure using the methods of Weir (1949) utilising the methods of establishing urinary nitrogen excretion of (Jequier et al., 1987).

Energy expenditure was thus established using the following equation:

$$EE = 3.941 \times \dot{V}O_2 + 1.106 \times \dot{V}CO_2 + 2.17 \times \text{Nitrogen Excretion}$$

2.6.6 Measurement of Resting Metabolic Rate

Measurements of resting metabolic rate (RMR) were obtained in accordance with the guidelines for best practice set out by Compher and colleagues (2006). Participants were asked to lie supine in the laboratory (which was maintained between 20-25 ˚C) for a period of 15 minutes prior to any measurement, during this time procedures were explained fully to the participant. Upon completion of the quiet rest period, participants were then familiarised with the mouthpiece and breathing apparatus, which also acted to flush atmospheric air from the tubing of the system. Four expired air samples of five minutes duration were then obtained. These samples were then analysed as previously described, with three measured daily energy expenditures within 100 kcal agreement deemed acceptable variation. The mean of the values within 100 kcal were then taken to represent RMR. If the first 4 measurements obtained did not provide samples of acceptable agreement, then additional measures (up to a maximum of six samples) were taken until the criteria described above were fulfilled.

2.6.7 Diet Induced Thermogenesis

For establishing diet induced thermogenesis (DIT) during laboratory visits, 5 minute expired gas samples were obtained hourly, after meals (for 3 hours post-breakfast and 3 hours post-lunch), in a semi-supine position following 5 minutes of complete rest. Diet induced thermogenesis was calculated as the mean energy expenditure over each 3 hour period of measurement minus the resting energy expenditure from the corresponding lab visit (i.e at baseline on the same day).

2.6.8 Dual Energy X-Ray Absorptiometry

Dual Energy X-Ray Absorptiometry (DEXA) scans were performed (QDR, Discovery W, Hologic, UK) prior to and after the free-living intervention. Prior to each scan, the device was calibrated using a quality control material provided by the manufacturer. Scans were normally performed at ~7.30 ± 1 h with participants overnight fasted, having consumed 568 mL of plain water upon waking and having voided immediately prior to scans. Participants were scanned in light clothing (i.e shorts for men and athletic clothing for women). Participants were positioned centrally on the scanning bed; with feet spread apart and hands placed in a mid-prone position such that there was a gap between the arms and trunk. This allowed regions of interest to be defined by an operator using the software provided by the manufacturer (QDR for Windows, Hologic, UK). The device operator was the same for both scanning occasions, with the same individual analysing participants' scans pre- and post-intervention. In certain cases due to availability of the scanning equipment, participants were scanned following the OGTT on Phase 2 trials. In these instances participants were scanned at the same time on both occasions due to the observed effects upon body composition of daily activities and food ingestion (Nana et al., 2011). Scans were analysed for assessment of total fat mass, lean body mass, and percentage body fat. Fat mass index was calculated as fat mass in kilograms estimated by DEXA divided by height in metres squared, with classification of obesity status according to the criteria established by Kelly and colleagues (2009).

2.6.9 Anthropometry

Height was measured and recorded to the nearest 0.1 cm using a fixed stadiometer (Holtain Ltd, UK). Body mass was measured post-void in light clothing (i.e shorts for men and athletic clothing for women) to the nearest 0.1 kg using electronic scales (Tanita Corporation, Japan). Waist circumference was taken as the midway point between the lowest rib and the top of the iliac crest, with hip circumference taken as the widest part of the buttocks. For both of these measures, measurements were obtained in triplicate, with an acceptable range of 1 cm for the three measurements. If this was not achieved in the first three measurements additional measurements were obtained until this criteria was met, with the mean of the three observations within 1 cm used. Sagittal abdominal diameter was measured using a

manual abdominal calliper (Holtain Ltd, UK), with participants supine and upon gentle expiration, at the level of the umbilicus. Three measures were obtained, with the mean of these measures used. All anthropometric measures were obtained by the same experimenter pre- and post-intervention for the same participant.

2.6.10 Adipose Tissue Biopsies

Subcutaneous abdominal adipose tissue biopsies were carried out under local anaesthetic. The area to be biopsied was first cleaned superficially using Videne (Ecolab, UK) and then anaesthetised using 1 % Lidocaine (Antigen Pharmaceuticals, UK) injected in a fan pattern to the subcutaneous adipose tissue parallel to the umbilicus using a 27G needle. Following 5 minutes to allow the anaesthetic to take effect, a 14G needle was then attached to a 60 mL syringe containing ~5 mL of 0.9 % Sodium Chloride infusion (B.Braun, UK). The plunger was removed to create a vacuum which was maintained throughout the biopsy. The needle was then inserted into the anaesthetised area and rocked gently for ~2 minutes to yield ≥ 500 mg of adipose tissue. Once sufficient tissue was removed, pressure was immediately applied to the biopsy site to minimise bruising.

2.6.11 Oral Glucose Tolerance Test

Participants consumed a glucose solution, comprised of 140 g of an orange flavoured high energy carbohydrate supplement (Polycal, Nutricia, UK) providing 75 g of glucose, diluted with water to a volume of 200 mL. In addition, participants consumed a further 100 mL of water from the same glass after they had consumed the glucose solution to ensure all of the glucose solution had been consumed and secondly, to allow the participant to clear any remaining glucose solution from the mouth. Following complete ingestion of the glucose solution, 5 mL blood samples were obtained every 15 min hereafter, with the final sample drawn at 120 min.

2.6.12 Insulin Sensitivity Indices

Analysis of the OGTT results included calculation of the incremental area under the curve (iAUC) for insulin and glucose concentrations according to the method recommended by Wolever (2004). Both the homeostatic model assessment of insulin resistance (HOMA-IR) (Matthews et al., 1985) and composite index of

insulin sensitivity (C-ISI) (Matsuda and DeFronzo, 1999) indexes were calculated using the plasma glucose and serum insulin concentrations from the oral glucose tolerance tests. These were calculated using the following formula:

HOMA-IR = ((Fasting Glucose ($mmol \cdot l^{-1}$) x Fasting Insulin ($\mu IU \cdot ml^{-1}$)) /22.5

C-ISI = 10000/SQRT ((Fasting Glucose ($mg \cdot dl^{-1}$) x Fasting Insulin ($\mu IU \cdot ml^{-1}$)) x (Mean Glucose over OGTT ($mg \cdot dl^{-1}$) x (Mean Insulin over OGTT ($\mu IU \cdot ml^{-1}$))

2.6.13 Appetite Sensations

Paper based, 100 mm visual analogue scales were used to assess subjective appetite. Participants marked on 100 mm vertical lines their response to questions assessing desire to eat, hunger, fullness and prospective consumption with anchor terms on each end of the scale (e.g not at all hungry vs as hungry as I have ever felt). Higher scores were indicative of greater sensations. A composite hunger score (Anderson et al., 2002) was calculated as the following:

(Desire to Eat + Hunger + (100-Fullness) + Prospective Consumption) /4.

2.7 Free-Living Measurements

2.7.1 Diet Analysis

For the analysis of energy intake, participants maintained weighed dietary records. Weighed records have been shown to be a more valid measure of energy intake than food dietary recall methods (Martin et al., 2002). Weighing of foodstuffs also negates the potential error in estimation of weight as Gittelsohn and colleagues (1994) have reported lower accuracy of weight estimates in foods of high volume and low weight. Participants maintained these records for a 7-day period, allowing an accurate representation of energy intake, as previous work has shown variation between weekday and weekend energy intake (Whybrow et al., 2008). This also allowed an analysis of measured energy balance, as this period was simultaneous with a 7-day period of physical activity energy expenditure measurement.

Details for individual food items were, wherever possible, obtained directly from manufacturers information on composition. These foods were then added to a database of "custom" food items that was accessible within the dietary analysis software for reference by the investigators. In instances where this was not possible (as information on brand was not provided or composition data was not available), or food was fresh (e.g fruits and vegetables) then foods were analysed using the standard food database information of Compeat Pro 5 diet analysis software (Nutrition Systems, UK). This programme utilises a UK integrated database based upon McCance and Widdowson's 6th Edition of The Composition of Foods (McCance et al., 2002).

Total energy intake and the percentage of energy intake from carbohydrates, fats, protein and alcohol were calculated. Additionally, the proportion of carbohydrates in participants' diets composed of sugars and starch were calculated as well as the proportion of saturated fat. Diets were analysed for each 24 hour period and also for pre- and post- 12:00. For analysis of feeding frequency, meals were defined as > 300 kcal intake, and distinct from another meal by > 45 minutes, with snacks defined as other feeding occasions (de Castro, 1994a).

It is reasonably well established that food records are liable to error, particularly underreporting (Poslusna et al., 2009). While there is much literature pertaining to the assessment of dietary records for feasibility (Black, 2000b; Goldberg

et al., 1991), in the present work previously proposed cut offs were not employed. While this may introduce some error due to unidentified underreporting, Black (2000) has identified that cut offs have little application on an individual basis. Additionally, weight change is one of the potential outcomes in the current work; therefore excluding dietary records on the basis that long term weight stability would not be maintained is not appropriate as this may be an outcome of the intervention period.

2.7.2 Continuous Glucose Monitoring Systems

Continuous glucose monitors (CGMS) were used to assess free-living glucose concentrations (CGMS® iPro, Medtronic MiniMed, USA). The device chosen has been previously shown to provide acceptable clinical accuracy in ≥ 95 % of measurements in type 1 diabetics (Sachedina and Pickup, 2003; Gross et al., 2000) as defined by the Clarke error grid which classifies the agreement between a glucose measure and a reference value (Clarke et al., 1987). Using this methodology, the two values are plotted to establish if the two measures are considered clinically accurate (Zone A-within 20% of criterion) or the difference is a benign error (Zone B) that would not result in the absence of necessary clinical intervention or inappropriate treatment. Anything outside of these two zones are not considered clinically acceptable. In a pilot study of accuracy in healthy subjects during daily activities it has been suggested that there was no statistically significant difference between CGMS and fingerprick data (Derosa et al., 2009), although this does not necessarily indicate good agreement.

One of the main differences between measured interstitial glucose concentrations and blood glucose is a lag in changes of interstitial glucose (Dye et al., 2010; Mazze et al., 2009). Indeed, lag of interstitial glucose concentrations has been reported in type 1 diabetics consuming two liquid meals over an 8 hour period (Boyne et al., 2003). Cheyne and colleagues (2002) investigated controlled hypoglycaemia (2.5 $mmol \cdot l^{-1}$) in healthy individuals and reported that the detection of the return to euglycaemia after hypoglycaemia was delayed by an average of 26 minutes, although the overall agreement with blood glucose was good with a mean absolute error of 7 ± 15 %.

The reproducibility and validity of the device for identifying hypoglycaemia in free-living type 1 diabetics has been described by Hoi-Hansen and colleagues (2005). They report that in participants fitted with two CGMS devices that when one device was recording a hypoglycaemic episode ($\leq$ 2.2 mmol·l^{-1}) in 91 % of occasions the other device was either also registering an episode (54 %) or interstitial glucose of >2.2-3.5 mmol·l^{-1} (37 %). When comparing CGMS results with a limited number of self-monitored blood glucose readings of hypoglycaemia 94 % were again either registering hypoglycaemia (53 %) or in the range of >2.2-3.5 mmol·l^{-1} (41 %).

Due to the nature of the device, the CGMS device has been predominantly validated in type 1 diabetics and with a focus on clinical accuracy (as described above). There is a lack of validity and reliability data in normal populations during free-living. In particular, there is a paucity of data in healthy, obese populations describing the use of this device and reliability and validity in obese individuals. Future research should also focus on establishing normal ranges in obese, healthy individuals as has been established in normal weight individuals (Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group, 2010; Hill et al., 2011).

Devices were employed as follows; electrochemical sensors utilising glucose oxidase were inserted into the subcutaneous abdominal tissue of participants using a lancing device provided by the manufacturer. This was then connected to a monitor secured in place by adhesive plasters (IV3000, Smith and Nephew, UK). The CGMS monitor took measures of interstitial glucose every 5 minutes, and was worn for 3-4 days. The indwelling sensor and monitor were replaced at this point, with a total wear time of 7 days. The monitors were retrospectively calibrated against capillary fingerprick glucose readings (Freestyle Lite, Abbott Diabetes, UK) obtained 4 times a day prior to meals using software provided by the manufacturer (Solutions Software for CGMS iPro version 2.2a, Medtronic Minimed USA). This is relevant as Buckingham et al (2006) report lower accuracy of calibration during periods of high rates of blood glucose change potentially due to the lag in interstitial blood glucose (Dye et al., 2010; Boyne et al., 2003). Participants were blinded to the readings of the fingerprick glucose monitors to prevent any behavioural adaptation to the perception of blood glucose level acceptability.

Participants maintained logs of waking and sleeping times during the two weeks of free-living monitoring, the results obtained from these logs were used to divide the day into periods from waking to 12:00, 12:00 to sleeping and sleeping. For these defined periods as well as the day as a whole, CGMS data for glucose was analysed for mean, standard deviation and coefficient of variation.

2.7.3 Combined Heart Rate Accelerometry

For the measurement of physical activity energy expenditure a combined heart rate-accelerometry monitor was used (Actiheart®, Cambridge Neurotechnology, UK). The Actiheart® is a non-invasive monitor, weighing less than 8g. Participants wore the monitors for 9 days (providing 7 full days of measurement) mounted on the chest using adhesive electrode pads (3M, UK) and were informed to wear the monitors continuously, only removing the monitors for washing or water based activities. This device has been shown to be reliable and valid (Brage et al., 2005) and able to accurately estimate energy expenditure in low to moderate intensity activities (Thompson et al., 2006). The monitor was used in the group calibration setting using participants' sleeping heart rate and gender (Brage et al., 2007). Due to the intensive nature of the experimental protocols involved for participants and substantive time commitment it was not feasible to include an individual calibration of these devices. This was primarily as for some participants the exercise involved in individual calibration would have been unaccustomed and as participants began their measurement of physical activity intervention immediately after their baseline visit, and therefore some modification of physical activity habits during this period may have occurred as a result. Due to the already heavy participant burden it was not deemed practical to include an additional laboratory visit for an exercise test to be conducted prior to beginning the free-living intervention.

Unlike many hip mounted accelerometers this device allows for greater wear time and therefore a more accurate picture of physical activity as there is less error due to bias from imputing of data (Catellier et al., 2005). Participants also maintained physical activity diaries, providing contextual information about patterns of wear. Where participants removed the monitor and reported increased physical activity (i.e

swimming) energy expenditure was interpolated using the Compendium of Physical Activities (Ainsworth et al., 2011).

2.7.4 Data Handling

Whilst the use of physical activity monitors is widespread in research settings, there have not been clear guidelines set out for many parameters such as duration of monitor wear (Matthews et al., 2012) or in fact what constitutes a valid day of measurement (Paul et al., 2008). This is in some respects understandable, as decisions relating to these key outcomes will be study specific (Heil et al., 2012). A particular weakness of the literature pertaining to the use of Actiheart® is a lack of information relating to the handling of data after collection. Considered in detail subsequently are the methods and rationales for dealing with the processing of physical activity traces obtained.

During wear there are occasions that the Actiheart® temporarily loses heart rate signal. During these occasions the monitor uses "recovered" HR observations and gives a 75% weighting to accelerometry based estimates of energy expenditure. While this is a pragmatic and practical approach to obtaining the greatest number of measures possible there are two main issues associated with this approach. Firstly, experiences from our laboratory indicate the "recovered" HR would seem to underestimate HR compared with what would be expected based upon the corresponding accelerometry data and activity diaries (see Figure 2.2 below). It would seem to follow that electrical conduction with the chest is reduced potentially when the unit is knocked, or during periods when the electrode pads may be less secure (i.e sweating). Therefore, the HR based component of energy expenditure would tend to be underestimated.

The strength of the energy expenditure estimates made by the device is based upon the combination of both the measures of heart rate and accelerometry (Brage et al., 2004). It is also particularly pertinent that investigations using Actiheart® have found that the accelerometry only based estimates when compared with other prediction models were the least predictive of energy expenditure (Crouter et al., 2008; Spierer et al., 2011). Therefore, in the present work it was decided that due to the potential modification of the energy expenditure estimates using this data that a criteria

of no greater than 22.5% of a given 24-hour period could be comprised of "recovered" data.

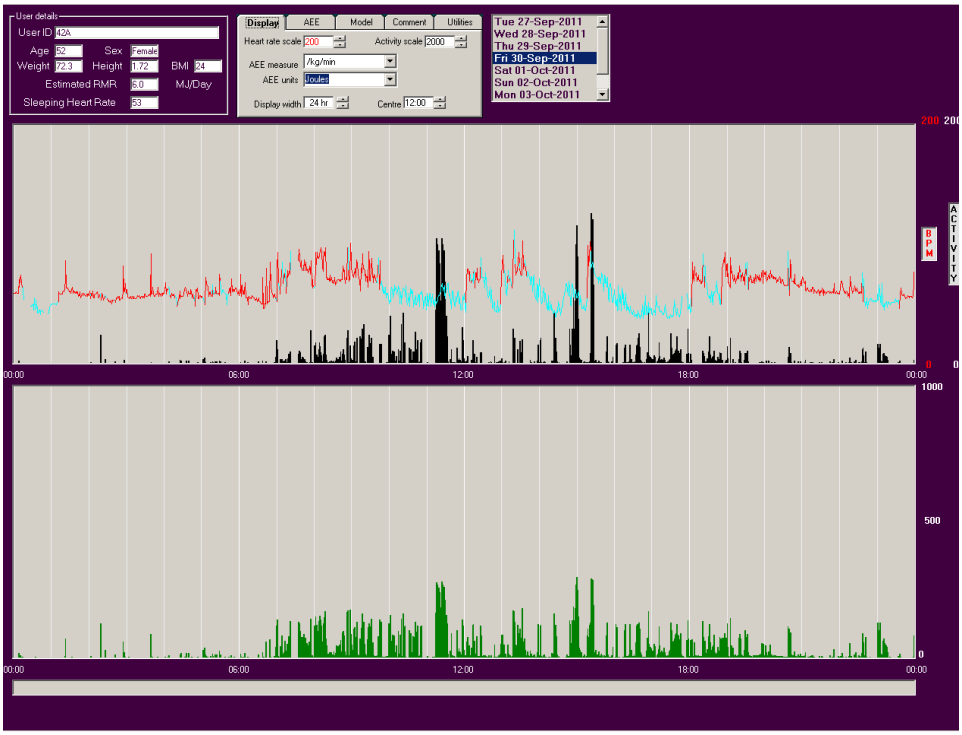

Figure 2.2: Example of "recovered" HR data from the Actiheart® device. Measured acceleration is displayed as the black bars, while heart rate is displayed as the red trace, "recovered" HR data is displayed in blue. The estimated energy expenditure is displayed as green bars in the bottom panel.

Due to the convenient nature of the device, potential wear time of the monitor is high relative to accelerometers. Therefore, for a day of wear to be classified as valid the percentage of time not lost (i.e through non-wear not including time accounted for in water based activity) was set at 90% of a 24 hour period. This figure was selected to minimise the potential error by imputation of data (Catellier et al., 2005). For each day where there was a proportion of lost data, the measured data was transformed to a 24 hour equivalent energy expenditure estimate.

The determination of the number of days required to assess physical activity in accelerometers has been considered at length by Matthews et al (2002). The authors propose a monitoring period of 3-4 days to achieve 80% reliability in activity counts. In the present work an acceptable number of valid (as defined by fulfilling of the two criteria set out above) days of monitoring was ≥4 days, to include one weekend day, due to potential for differences in weekday to weekend activity patterns (Matthews et

al., 2002). Participants were only included in the physical activity analysis if both weeks of recording met the criteria outlined. The criteria for inclusion in data analysis are summarised in Figure 2.3.

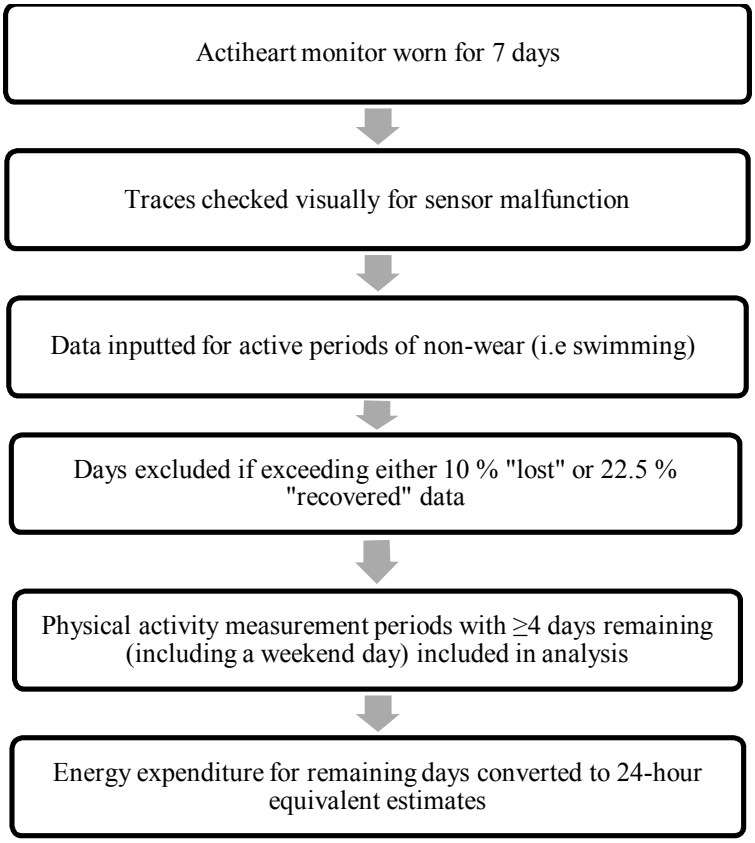

Figure 2.3: Schematic of the data analysis process for physical activity data.

The physical activity data obtained was analysed for total physical activity energy expenditure, as well as energy expenditure according to intensity for both pre- and post-12:00. This was classified as energy expenditure expressed as multiples of measured resting metabolic rate ((i.e metabolic equivalents, METs (Ainsworth et al., 2000)). These intensity domains for physical activity were based upon previously established cutoffs (Haskell et al., 2007; Pate et al., 2008).

2.7.5 Lifestyle Maintenance Data

Lifestyle maintenance/monitoring was applied to any willing participant eligible for randomisation into the breakfast/fasting treatment groups (thus broadly equivalent to the main study population) but unable to commit to the main study for other reasons (e.g. impossible to schedule trials in the required time-frame, unwilling

to provide tissue samples, *etc*.). Monitoring free-living responses in the absence of any intervention therefore provides context regarding the extent to which those free-living measures alone (i.e. dietary and physical activity monitoring) may impact typical energy intake (2391 ± 626 kcal·d^{-1}), physical activity levels (1230 ± 603 kcal·d^{-1}) and change in body mass body mass over 6 weeks (-0.09 kg; 95% CI = -0.8, 0.6) under conditions where diet and physical activity are monitored as applied in the main experiment.

Chapter 3: Morning fasting is incompletely compensated for at an *ad libitum* lunch and causes altered second meal metabolism during the afternoon in lean adults

3.1 Introduction

Regular breakfast omission is associated with many negative health outcomes, including greater risk of obesity (Ma et al., 2003; Horikawa et al., 2011), prospective weight gain (Purslow et al., 2008), type 2 diabetes (T2D) (Mekary et al., 2013; Mekary et al., 2012) and coronary heart disease (Cahill et al., 2013b). These detrimental associations are particularly relevant as Reeves et al. (2013) have suggested that 30% of British adults occasionally skip breakfast. As increased adiposity is due to chronic positive energy balance; laboratory studies have understandably explored a possible role of daily breakfast in regulating energy intake (EI) and associated metabolic responses. However, the major focus of previous research on this topic has been to contrast breakfasts of varied quantity or composition, for example by manipulating macronutrient (Clegg and Shafat, 2010), fibre (Hamedani et al., 2009; Kim et al., 2009; Levine et al., 1989; Liljeberg et al., 1999), energy content (Martin et al., 2000) and/or glycaemic index (Rosen et al., 2011; Nilsson et al., 2008). In contrast, far less attention has been given to comparing the effects of breakfast consumption *versus* morning fasting upon acute energy intake.

Studies that have compared energy intake following morning fasting with a variety of breakfasts have yielded equivocal results, with both reduced (Gonzalez et al., 2013; Levitsky and Pacanowski, 2013) and similar (Astbury et al., 2011) daily energy intakes observed with several permutations of fixed intake and *ad libitum* feedings throughout the day. While some research has attempted to address the impact of morning fasting upon acute energy intake, to the authors' knowledge, only two studies have measured the hormonal and appetite responses to morning fasting and breakfast consumption followed by an *ad libitum* lunch (Astbury et al., 2011; Gonzalez et al., 2013). Whilst these studies provide valuable information regarding regulatory hormones and energy intake at an *ad libitum* lunch in response to breakfast omission, both of these designs employed a mid-morning preload (250 and 358 kcal)

that was consumed in both trials prior to the provision of the lunch. As a result, in both breakfast skipping conditions the participants had not undertaken unbroken fasting prior to eating the *ad libitum* lunch. The aforementioned design therefore provides an appropriate analogy with an individual that has skipped breakfast but then "snacked" prior to eating lunch, yet the hormonal and metabolic responses following an *ad libitum* lunch after unbroken morning fasting are yet to be determined.

The current study examines acute energy intake, appetite regulatory hormones and metabolic responses after extended morning fasting relative to a standardised breakfast. We hypothesise that morning fasting will result in incomplete EI compensation (i.e increased intake at lunch but not of an equivalent magnitude as breakfast intake) but greater appetite sensations and orexigenic hormone responses throughout the day.

3.2 Participants and Methods

3.2.1 Participants

Thirty five healthy, lean men (n = 14) and women (n = 21) aged 22-56 y took part in this study. Participants were recruited via local advertisement from South West England and were initially assessed for eligibility based upon a body mass index of 18-25 $kg \cdot m^{-2}$ and then later classified as lean based upon DEXA-derived fat mass indices of ≤ 7.5 $kg \cdot m^{-2}$ (men) and ≤ 11 $kg \cdot m^{-2}$ (women) (Kelly et al., 2009). The study was part of a wider randomised controlled trial (the Bath Breakfast Project). In accordance with the full eligibility criteria set out in Chapter 2, participants reported being weight stable (± 2% body mass within past 6 months) and adhered to a standard sleep-wake cycle (e.g no shift workers) and did not anticipate any change in lifestyle during the study period. Participants were free of metabolic disorders, with pre-menopausal female participants either menstruating regularly, or following their chosen contraceptive method (i.e pill, implant) for >6 months. Within the study cohort there was a mix of regular breakfast consumers (classified as >50 kcal intake within 2 hours of waking on ≥4 days of the week) and non-consumers. Characteristics of participants are presented in Table 3.1.

Written informed consent was obtained from all participants, with the Ethical Approval for the study obtained from the NHS Bristol Research Ethics Committee. The study is registered with Current Controlled Trials [ISRCTN31521726].

Table 3.1: Participant characteristics

Characteristic		
n		**35**
Age (y)		**36 (11)**
Body Mass (kg)		**67.9 (9.2)**
Body Mass Index (kg/m^2)		**22.7 (2.5)**
Fat Mass Index (kg/m^2)*	**All**	**5.7 (2.2)**
	Female	**6.7 (2.0)**
	Male	**4.1 (1.4)**
Habitual Breakfast Consumers (*n*)		**27**
Female (*n*)		**21**

*Fat mass index calculated as DEXA derived total fat mass divided by height squared. Data presented are Mean with (SD)

3.2.2 Study Design

Each participant undertook a randomised, counterbalanced crossover design involving two laboratory based feeding trials in the Human Physiology Laboratories at the University of Bath. These trials were separated by 3-28 days where participants resumed their habitual routine, with eumenorrheic women undergoing testing during the follicular phase of the menstrual cycle (3-10 days after the onset of menses) to standardise any effects of phase upon appetite hormones and energy intake between trials (Buffenstein et al., 1995; Lissner et al., 1988).

3.2.3 Protocol for Laboratory Visits

The protocol during experimental visits is displayed in Figure 3.1. Upon arrival at the laboratory at 08:00 ± 1 h, adherence to standardisation measures as described in Chapter 2 was confirmed verbally. Participants then voided and had body mass measured in light clothing (Seca 873, Vogel and Halke). On their first visit to the laboratory, following 20 minutes of quiet rest, resting metabolic rate was assessed in a supine position by repeated 5 minute expired gas samples over ~30 minutes, according to best practice (Compher et al., 2006). During the second visit to the laboratory, another resting sample was obtained for comparison with energy expenditure during that trial and to confirm the previously measured value from the first visit. A cannula was then inserted into an antecubital vein, with a baseline sample of 15 mL blood obtained and further samples acquired at regular intervals throughout the day. Participants were then provided with either a breakfast (to be consumed within 15 minutes) or asked to rest for the same duration, with blood samples taken at 15 minutes, 30 minutes and an hour post completion of the breakfast period. One hour after the breakfast/rest period, an expired gas sample was obtained for assessment of diet-induced thermogenesis. Blood and gas samples were then obtained hourly until 3 h post breakfast, at which point an *ad libitum* lunch was provided. The lunch period was of 30 minute duration. Samples of blood and expired gas were obtained hourly after completion of the lunch period for a further 3 hours, with the first samples obtained an hour after lunch was finished. Participants also completed visual analogue scales relating to hunger and appetite throughout the day. During the day participants remained sedentary and completed quiet activities such as reading, watching television

and typing. Full details of techniques and measurements employed are described in Chapter 2.

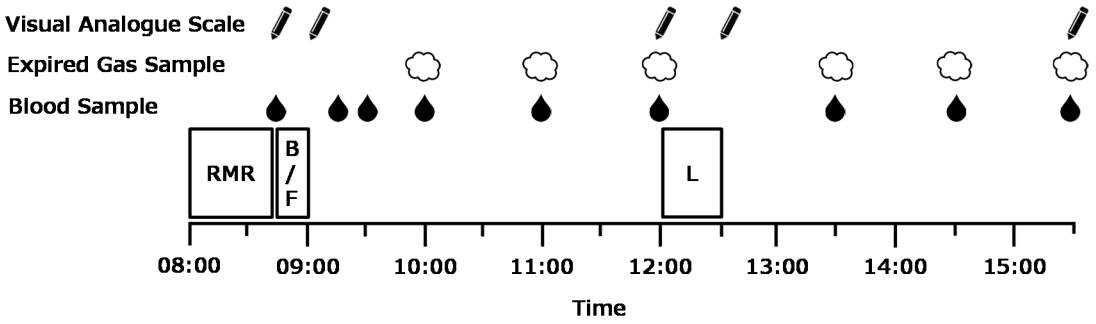

Figure 3.1: Schematic of experimental protocol. RMR=Resting Metabolic Rate, B=Breakfast consumption, F=Fasting, L=*Ad libitum* lunch

3.2.4 Breakfast

The breakfast consisted of Corn Flakes (Kellogg's), 2% Fat Milk (Sainsbury's), toasted white bread (Braces), margarine (I can't believe it's not butter) and fresh orange juice (Sainsbury's) and was based upon the breakfast provided by Chryssanthopoulos and colleagues (2004). Participants were given the choice of either white sugar added to cornflakes, or seedless raspberry jam (Sainsbury's) on their toast, or an iso-caloric combination of both. The overall percentages of energy from macronutrients in the breakfast were 70 % carbohydrate, 17 % fat and 13% protein. The breakfast was provided in quantities that contained 0.06 g carbohydrate per kcal of each individual participant's measured daily resting metabolic rate, resulting in an energy intake of 469 ± 57 kcal. Quantities of the items provided for a typical breakfast are illustrated in Appendix 1. Participants were first provided with cereal, then at 5 minute intervals toast and finally orange juice, with all of the breakfast consumed within 15 minutes to standardise any effects of eating rate upon appetite hormones (Kokkinos et al., 2010). During the fasting trial participants sat quietly for a matched time period.

3.2.5 *Ad Libitum* Lunch

Three hours post-breakfast participants were provided with an *ad libitum* lunch test meal consisting of 1 kg cooked (i.e wet weight) penne pasta (Sainsbury's) and tomato sauce (Ragu); prepared at a ratio of 1:1 uncooked mass. The overall percentages of energy from macronutrients for the lunch were 79 % carbohydrate, 14

% fat and 7 % protein. During their first trial participants were allowed *ad libitum* intake of plain water during lunch, this volume was subsequently replicated on their second visit. Participants were left alone during the lunch, with a recorded message played prior to beginning consumption "We ask that you continue eating until you have satisfied your hunger. The lunch will remain in front of your for at least 30 minutes, at which point the post-lunch timer will be started, although you will be allowed to continue eating if you are still hungry." Pasta was provided in a large bowl containing 1 kg of cooked pasta which was replenished every 10 minutes during the lunch period to minimise any visual feedback relating to consumption volumes, and prevent any tendency to completely finish the portion provided. The mass of pasta consumed during the lunch was recorded and energy intake was calculated using manufacturer's nutritional information.

3.2.6 Statistical Analysis

For single comparisons of two means (e.g energy intake at lunch), paired t-tests were employed. For comparison of time series variables that were measured over the course of the day in each treatment (e.g appetite hormones), repeated measures ANOVA were employed to identify interactions independent of deviations from a normal distribution (Maxwell and Delaney, 1990) but with Greenhouse-Geisser corrections applied to intra-individual contrasts for $\varepsilon < 0.75$, and the Huynh-Feldt correction applied for less severe asphericity (Atkinson, 2002). ANOVA results are described using the following terms: a main effect of trial refers to a difference of the overall means between the two conditions (i.e breakfast consumption and fasting trial days, irrespective of the time course within each day); a main effect of time refers to significant differences over the course of the testing day (i.e a change between time points within a trial day, irrespective of condition); and an interaction effect indicates that the time course of the measure was different dependent upon the trial being undertaken (i.e the time course varies between trial days).

For ANOVA, significant interaction effects were explored with *post-hoc* t-tests that compared the difference between specific time points in the two trials. Where multiple comparisons for a measure were conducted, these were corrected for potential inflation of type 1 error rate by using a Holm-Bonferroni stepwise adjustment (Ludbrook, 1998). For all statistical analyses, significance was accepted at $p \leq 0.05$.

Data are presented in text as mean ± standard deviation, figures display mean with normalised confidence intervals (nCI). These confidence intervals do not represent the variance around the mean for each time point (as would SD) but represent the comparison between the two trials, removing the inter-individual variation due to the fully paired nature of the experimental design (Loftus and Masson, 1994). In general, where these confidence intervals do not overlap by more than half the distance of one side of a confidence interval, these contrasts are likely to be deemed statistically significant using conventional hypothesis testing. All statistical analyses were conducted using IBM SPSS statistics version 22 (IBM, New York).

3.3 Results

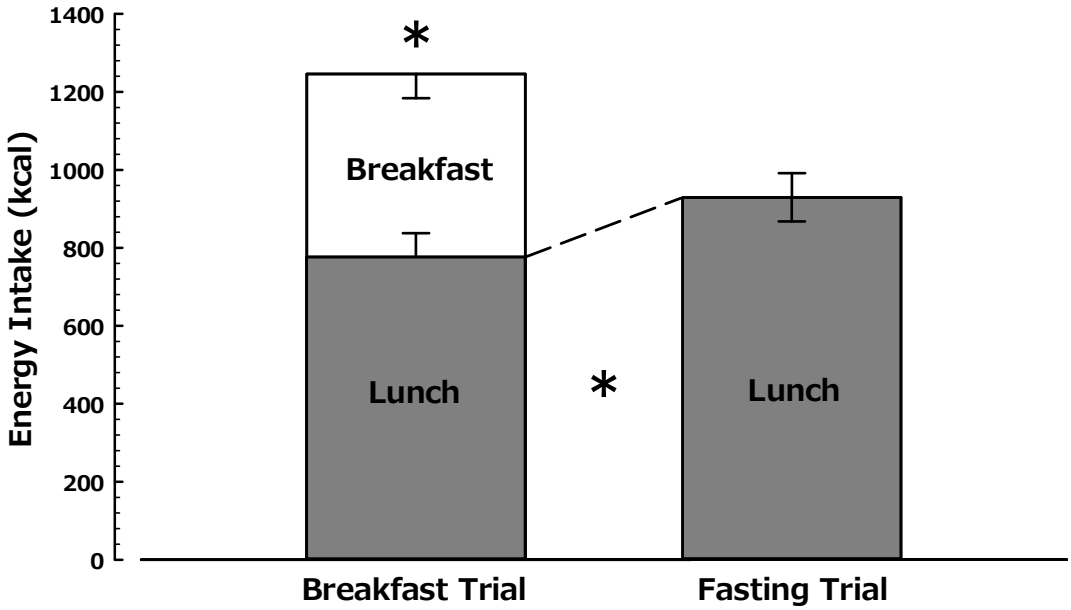

Figure 3.2: Energy intake during trials. In the fasting trial an asymmetric error bar is plotted. The positive portion of the error bar reflects the comparison between total intake during the fasting trial (i.e lunch only) and total intake on the breakfast trial, with the negative portion reflecting the comparison between lunches. An asterisk above a bar represents the comparison between the sum of the components of the bars, an asterisk between the bars represents the comparison between the specific component. * $p < 0.01$

3.3.1 Energy Intake

During the breakfast trial, participants consumed a prescribed breakfast of 469 ± 57 kcal (i.e variance proportionate to inter individual differences in RMR). During the *ad libitum* lunch, participants consumed 776 ± 349 kcal following breakfast but significantly more in the fasting trial (929 ± 317 kcal; $p < 0.01$). However, when the absolute energy intake during the breakfast trial was calculated (i.e breakfast + lunch), this was significantly more than during the fasting trial (1246 ± 380 *vs* 929 ± 317 kcal; $p < 0.001$). When comparing the energy intake at lunch in the two conditions, the additional energy intake at lunch of 153 kcal during the fasting trial accounted for ~33% of the prescribed breakfast.

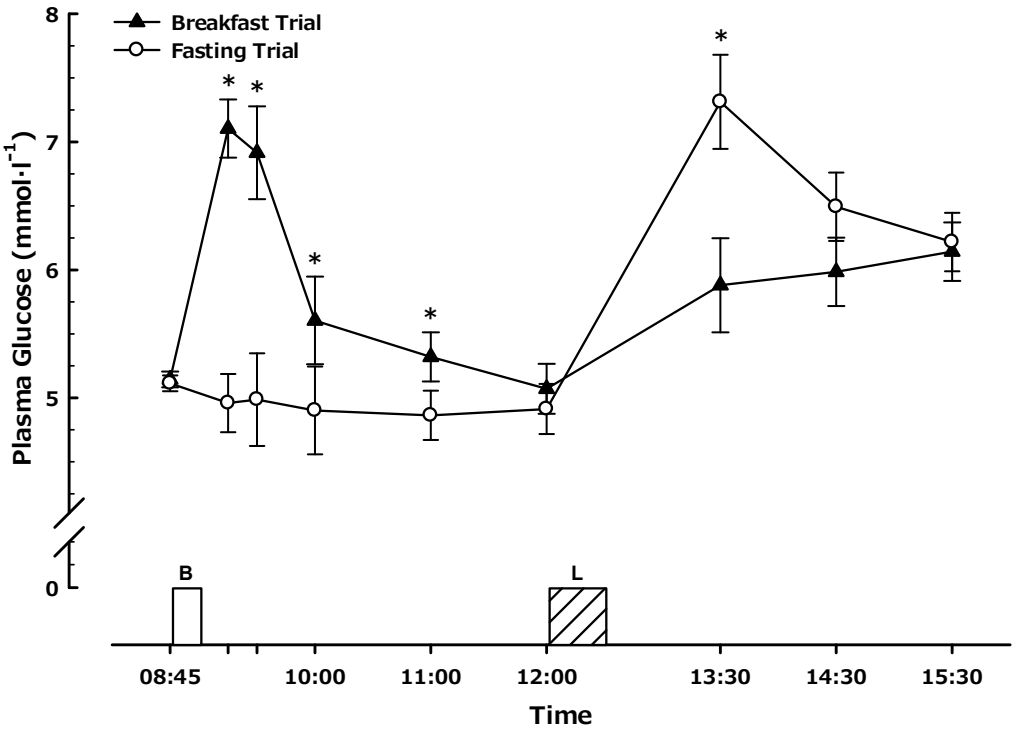

Figure 3.3: Glucose responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.03$ versus corresponding time point in other trial

3.3.2 Glucose

There were main effects of time, trial and a trial x time interaction for plasma glucose concentrations (all $p < 0.01$). Baseline glucose concentrations were not different between trials ($p = 0.80$, Figure 3.3), but were significantly greater following breakfast consumption until 2 hours post-breakfast (all $p < 0.03$). By 3 hours post-breakfast there was no difference in plasma glucose concentrations between trials ($p = 0.30$). Glucose concentrations were significantly greater 1 hour post-lunch in the fasting trial ($p < 0.01$). There was a strong tendency for greater concentrations in the fasting trial at 2 hours post-lunch (Breakfast Trial, 5.99 ± 0.75 mmol·l^{-1} *vs* Fasting Trial, 6.49 ± 1.02 mmol·l^{-1}; $p = 0.06$) but no difference between trials 3 hours after lunch ($p = 0.63$).

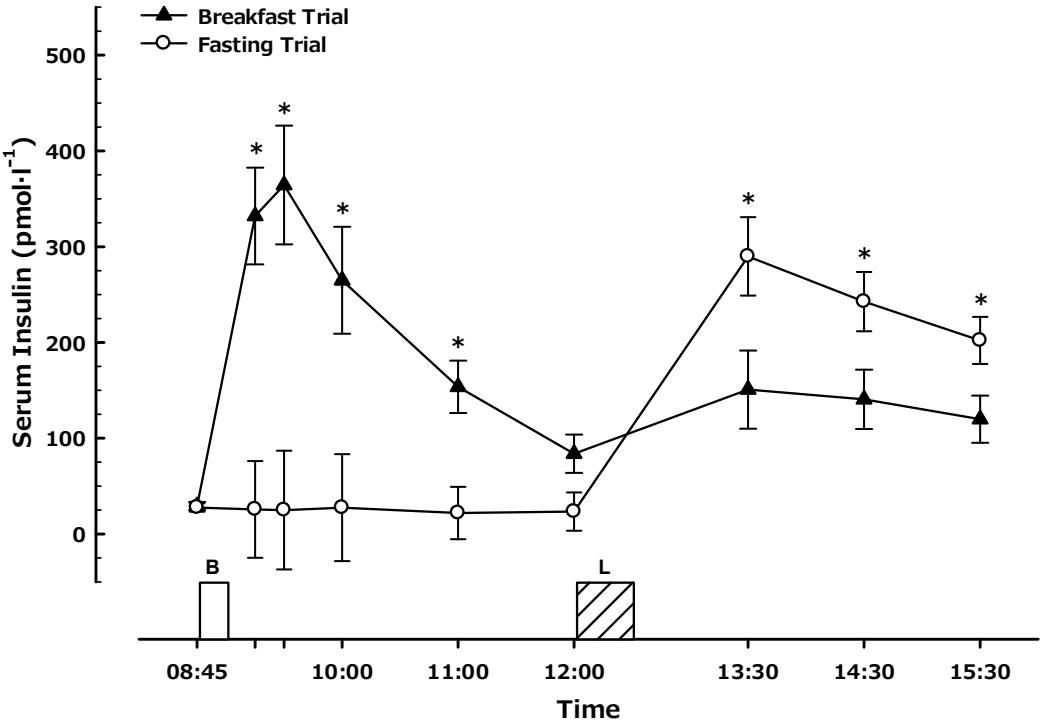

Figure 3.4: Insulin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

3.3.3 Insulin

There were main effects of time, trial and a trial x time interaction for serum insulin concentrations (all $p < 0.01$). Insulin concentrations were not different between trials at baseline ($p = 0.45$) but following breakfast consumption were significantly greater during the morning than during the fasting trial (all $p < 0.01$, Figure 3.4). Following lunch, insulin concentrations were significantly greater in the fasting trial than the breakfast trial (all $p < 0.01$).

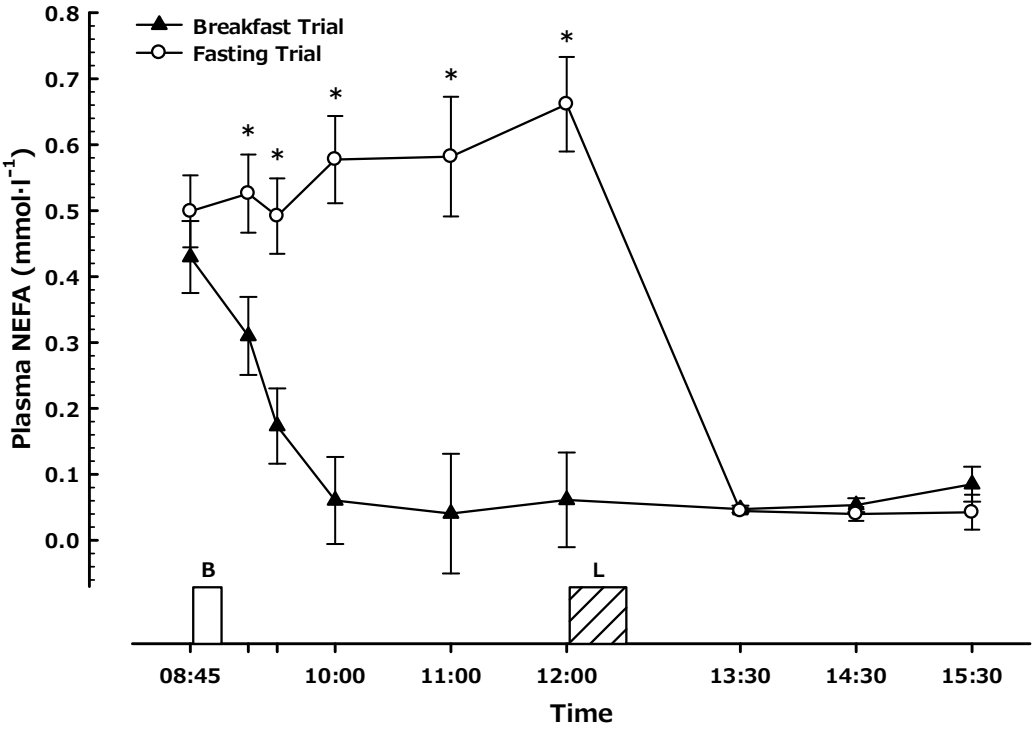

Figure 3.5: NEFA responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

3.3.4 NEFA

There were main effects of time, trial and a trial x time interaction for plasma NEFA concentrations (all $p < 0.01$). There was no difference in baseline NEFA concentrations in the two trials ($p = 0.25$, Figure 3.5). NEFA concentrations were lower for the 3 hour period following breakfast consumption (all $p < 0.01$). Following lunch there was no difference in NEFA concentrations (all $p > 0.05$).

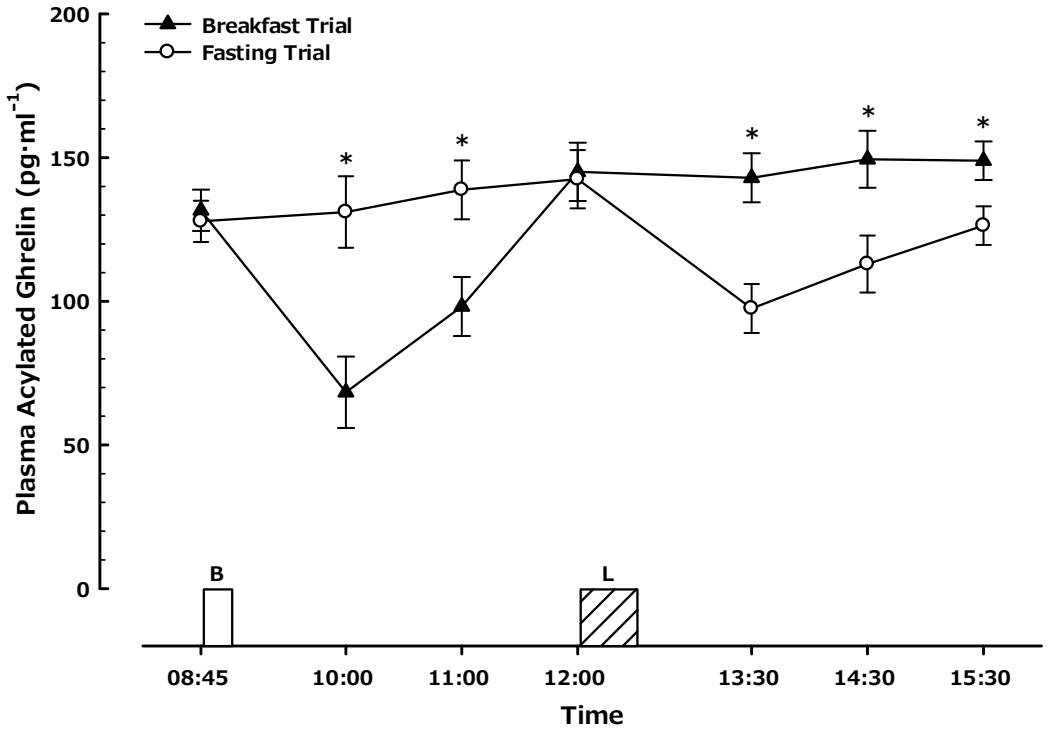

Figure 3.6: Acylated Ghrelin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

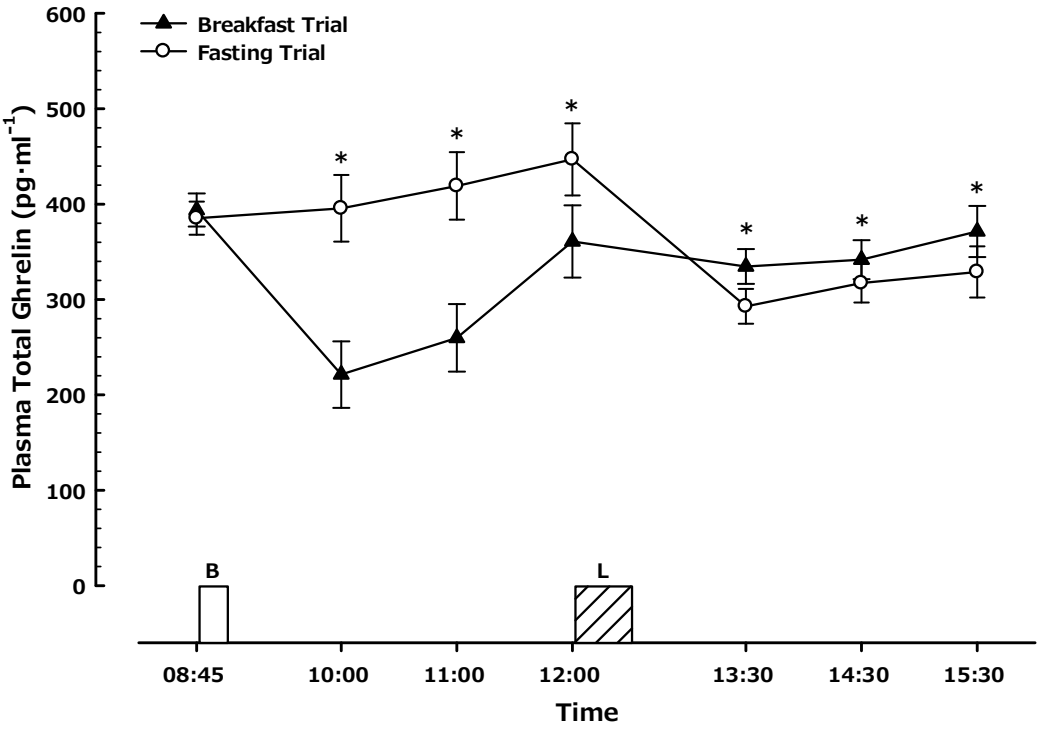

Figure 3.7: Total Ghrelin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p \leq 0.05$ versus corresponding time point in other trial

3.3.5 Acylated and Total Ghrelin

There were main effects of time and a trial x time interaction for plasma acylated ghrelin (both $p < 0.01$). Baseline concentrations of acylated ghrelin were not different between trials ($p = 0.79$). Breakfast consumption suppressed acylated ghrelin concentrations such that these were significantly lower 1 and 2 hours post-breakfast relative to the fasting trial (both $p < 0.01$, Figure 3.6) but there was no difference between trials at 3 hours post-breakfast (Breakfast Trial, 145 ± 83 $pg \cdot ml^{-1}$ *vs* Fasting Trial, 143 ± 73 $pg \cdot ml^{-1}$; $p = 0.72$). Acylated ghrelin concentrations were significantly greater than the fasting trial throughout the afternoon in the breakfast trial (all $p < 0.01$), with concentrations remaining above pre-lunch values during the breakfast trial, but lower than pre-lunch during the fasting trial.

There were main effects of time, trial and a trial x time interaction for plasma total ghrelin concentrations (all $p < 0.01$). Total ghrelin concentrations were not different between trials at baseline ($p = 0.57$). Following breakfast consumption, total ghrelin concentrations were suppressed resulting in significantly lesser concentrations than the fasting trial at all time points prior to lunch ($p < 0.01$, Figure 3.7). Following lunch, there was limited suppression of ghrelin in the breakfast trial such that total ghrelin concentrations at 1 hour post lunch were 85 % of baseline concentrations and rose throughout the afternoon reaching 94 % of baseline concentrations at 3 hours post-lunch. There was greater suppression of total ghrelin in the fasting trial after lunch, such that concentrations were significantly lower than breakfast throughout the afternoon (all $p \leq 0.05$).

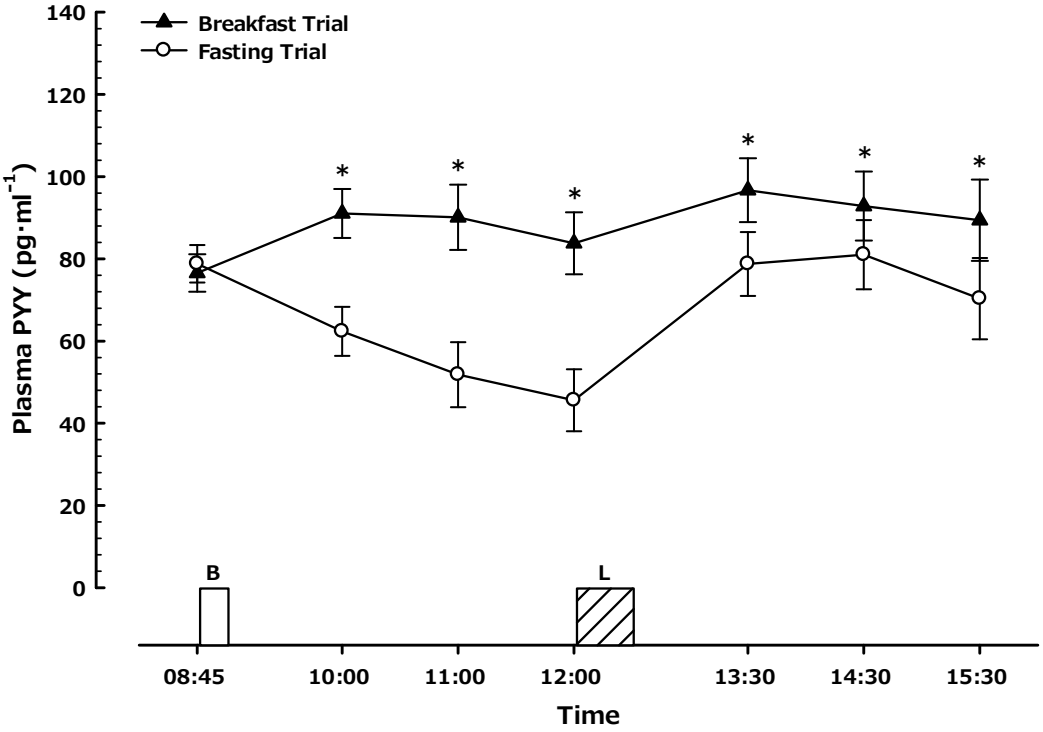

Figure 3.8: PYY responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.05$ versus corresponding time point in other trial

3.3.6 PYY

There were main effects of time, trial and a trial x time interaction for plasma PYY concentrations (all $p < 0.01$). Baseline PYY concentrations were not different between trials ($p = 0.35$, Figure 3.8). Significant differences between the trials at all time points prior to lunch were apparent for PYY concentrations ($p < 0.01$). At 1 hour post-lunch in the fasting trial PYY concentrations had returned to baseline levels of 79 ± 37 pg·ml^{-1}, whilst in the breakfast trial, PYY concentration peaked at 97 ± 46 pg·ml^{-1}, resulting in a significant difference between the two trials ($p < 0.01$). PYY concentrations remained greater in the breakfast trial than the fasting trial throughout the rest of the afternoon ($p < 0.05$).

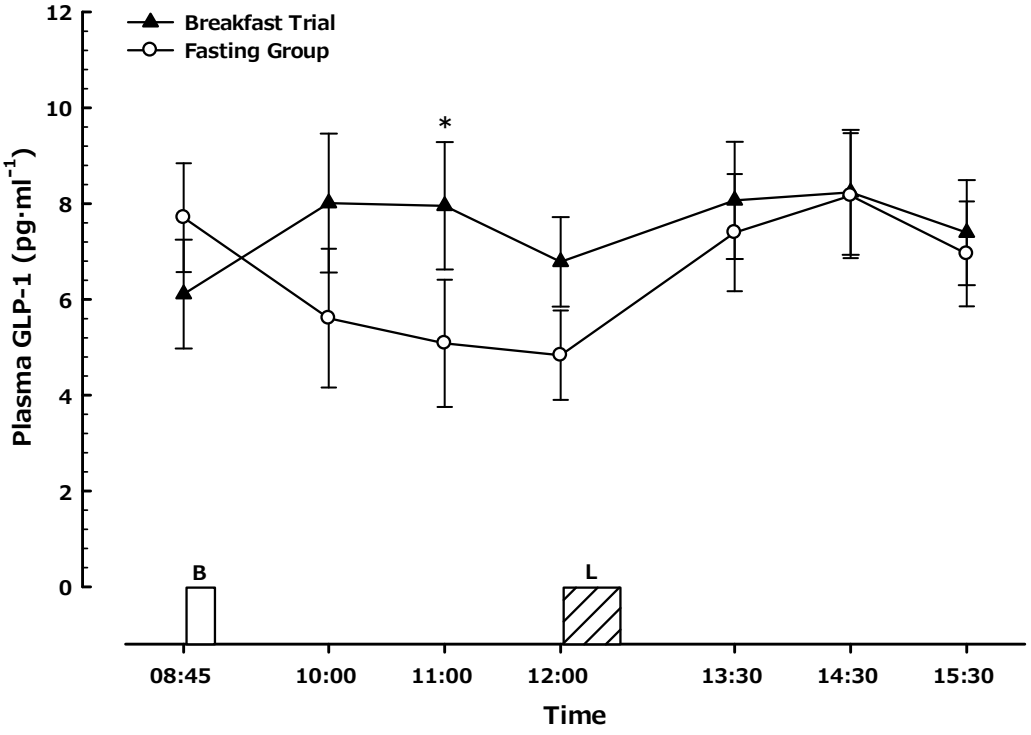

Figure 3.9: GLP-1 responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p = 0.02$ versus corresponding time point in other trial

3.3.7 GLP-1

There was a tendency for a main effect of time ($F = 2.27$, $p = 0.08$), but no effect of trial ($F = 2.89$, $p = 0.1$) for plasma GLP-1 concentrations. There was a trial x time interaction ($F = 3.09$, $p = 0.03$) for GLP-1 concentrations which are displayed in Figure 3.9.

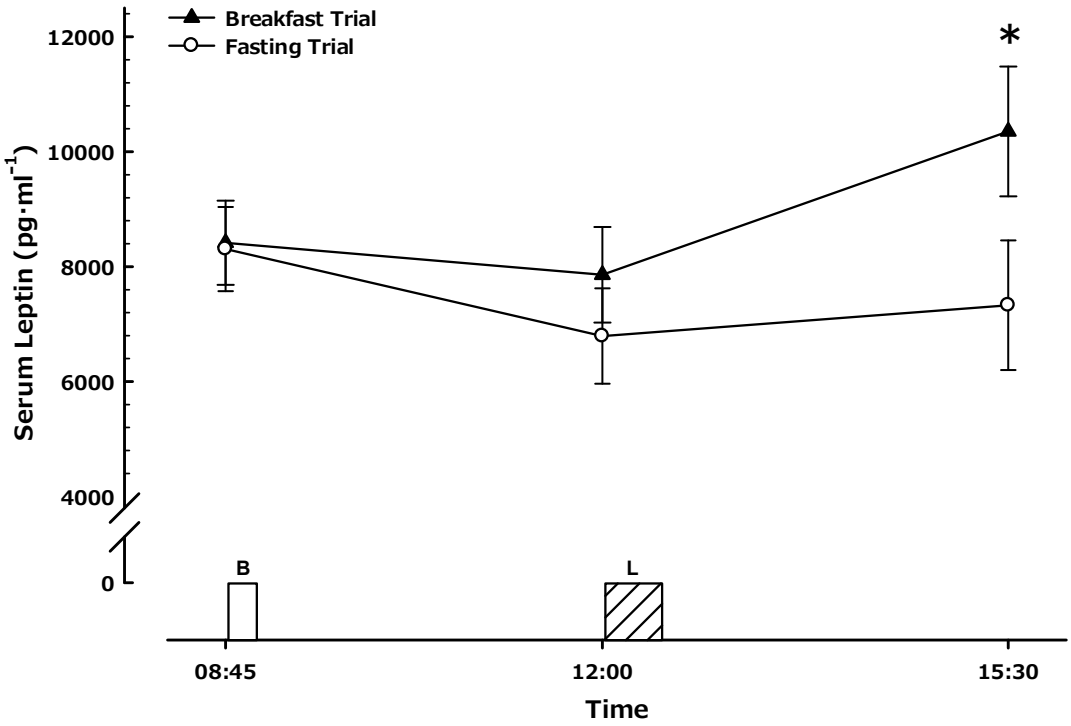

Figure 3.10: Leptin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

3.3.8 Leptin

Serum leptin concentrations in both trials are displayed in Figure 3.10. There were main effects of time, trial and a trial x time interaction for leptin (all $p < 0.02$). Baseline leptin concentrations were not different between trials ($p = 0.83$). In the breakfast trial, leptin concentrations were reduced from baseline at 3 hours post-breakfast, but this reduction was greater in the fasting trial, such that there was a trend for lower leptin concentrations in the fasting trial (Breakfast Trial, 7859 ± 7282 pg·ml^{-1} *vs* Fasting Trial, 6792 ± 6632 pg·ml^{-1}; $p = 0.07$). Three hours post-lunch, leptin concentrations had increased in the breakfast trial to a greater extent than in the fasting trial, resulting in a significantly greater concentration of leptin in the breakfast trial ($p < 0.01$).

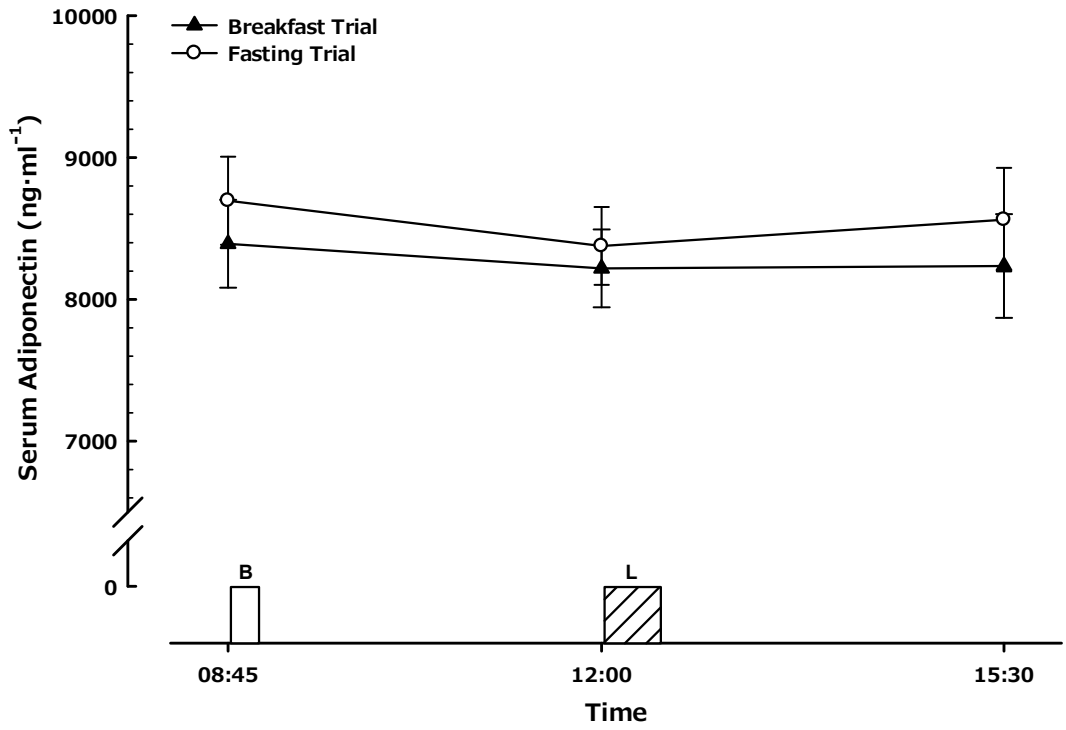

Figure 3.11: Adiponectin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch.

3.3.9 Adiponectin

There was a main effect of time for serum adiponectin concentrations ($F = 5.60$, $p < 0.01$). There were no main effects of trial ($F = 1.86$, $p = 0.18$) or an interaction effect apparent ($F = 0.47$, $p = 0.62$), such that adiponectin concentrations displayed similar responses in both trials (Figure 3.11).

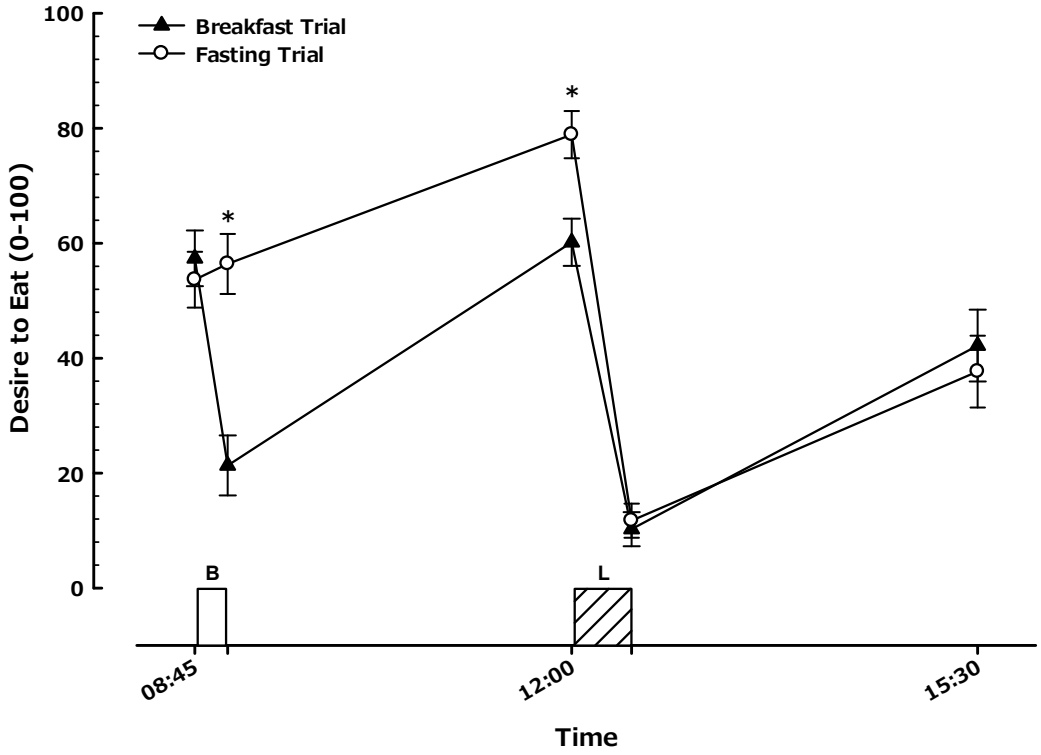

Figure 3.12: Desire to eat during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

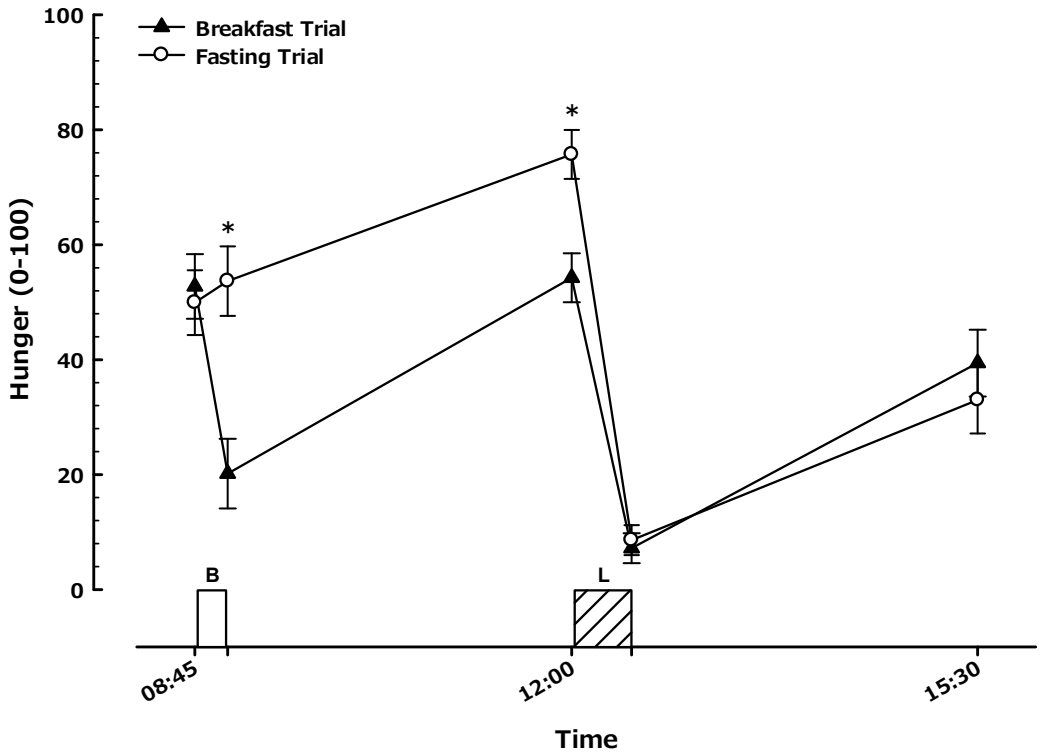

Figure 3.13: Hunger during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

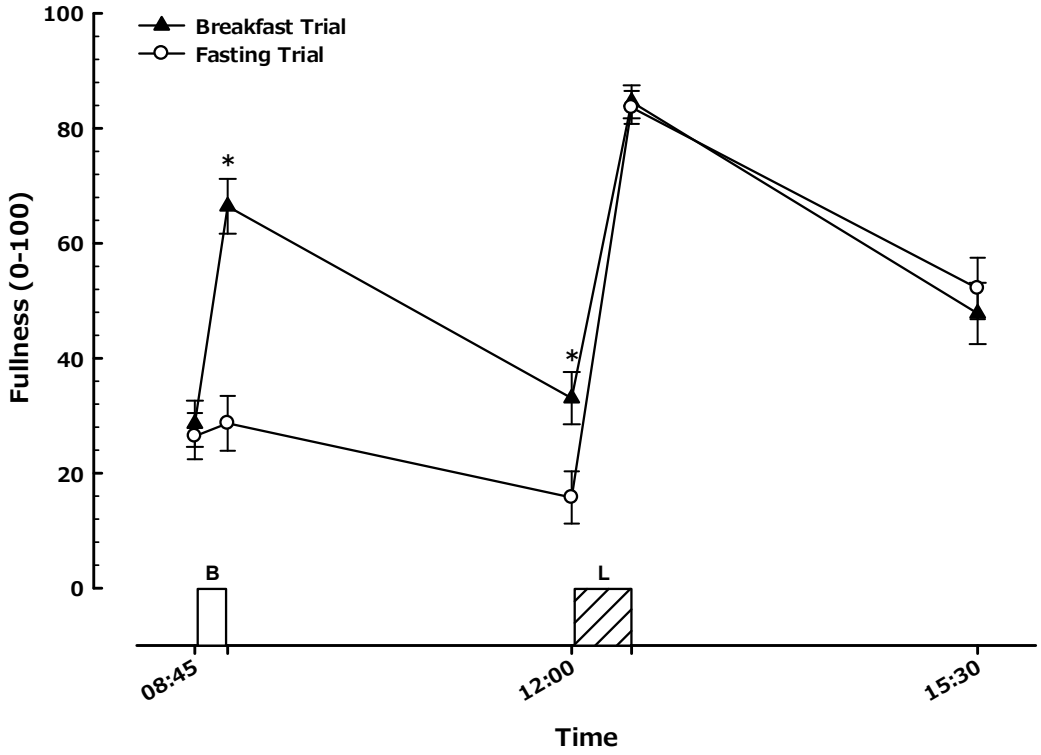

Figure 3.14: Fullness during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

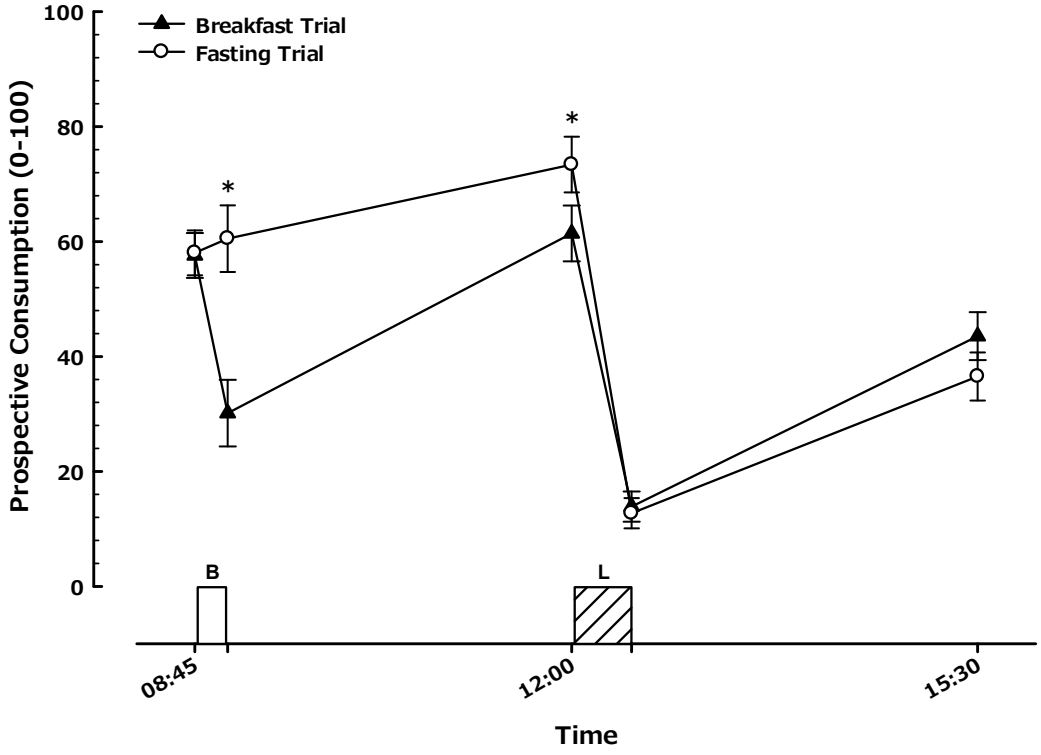

Figure 3.15: Prospective consumption during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

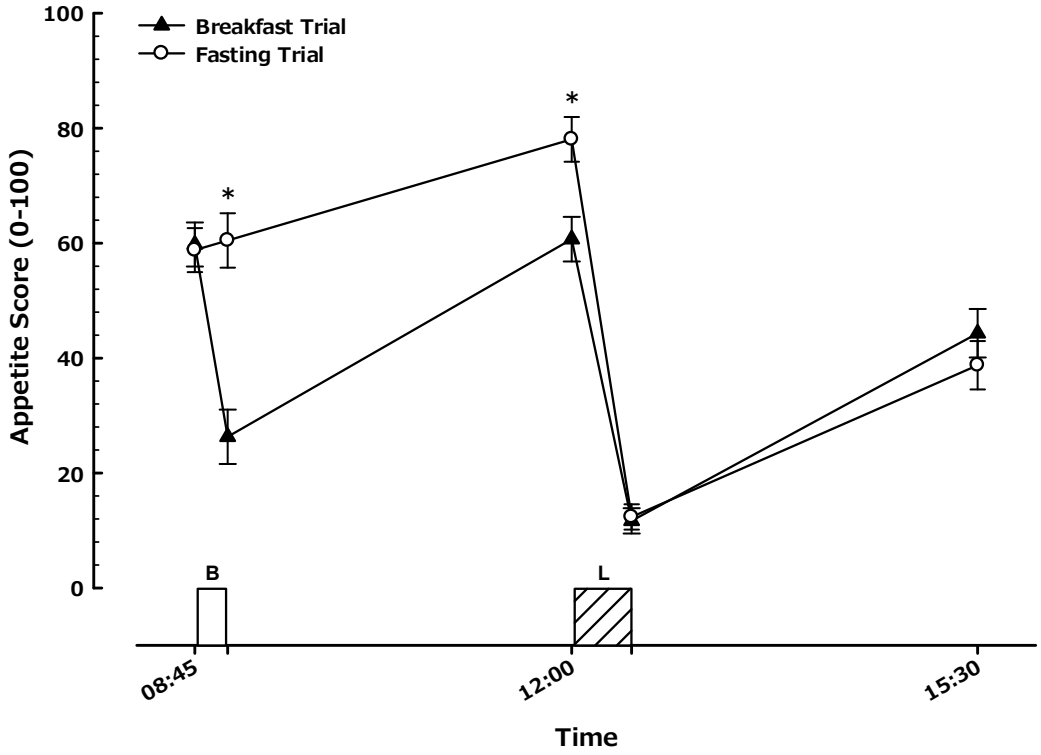

Figure 3.16: Appetite score during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

3.3.10 Subjective Appetite Ratings

Results from the hedonic scales provided to participants throughout the day are displayed in Figures 3.12-16. There were no differences at baseline for any of the measures (all $p > 0.2$). Desire to eat, hunger and prospective consumption all followed very similar patterns throughout the day. Immediately after breakfast consumption there was a reduction in all three measures such that there was a highly significant difference between all three measures compared with the fasting trial (all $p < 0.01$). Immediately prior to lunch these appetite sensations were greater in the fasting than breakfast trial (all $p < 0.01$). Following the consumption of the *ad libitum* lunch all three measures decreased to a nadir with no significant difference between trials (all $p > 0.4$). Three hours after completion of lunch there was no difference between trials for any of the measures (all $p \geq 0.1$). The sensations of fullness followed a similar, but opposite, pattern throughout the day (Figure 3.14). The composite appetite score

combining the hedonics described above shows a similar pattern as desire to eat, hunger and prospective consumption (Figure 3.16).

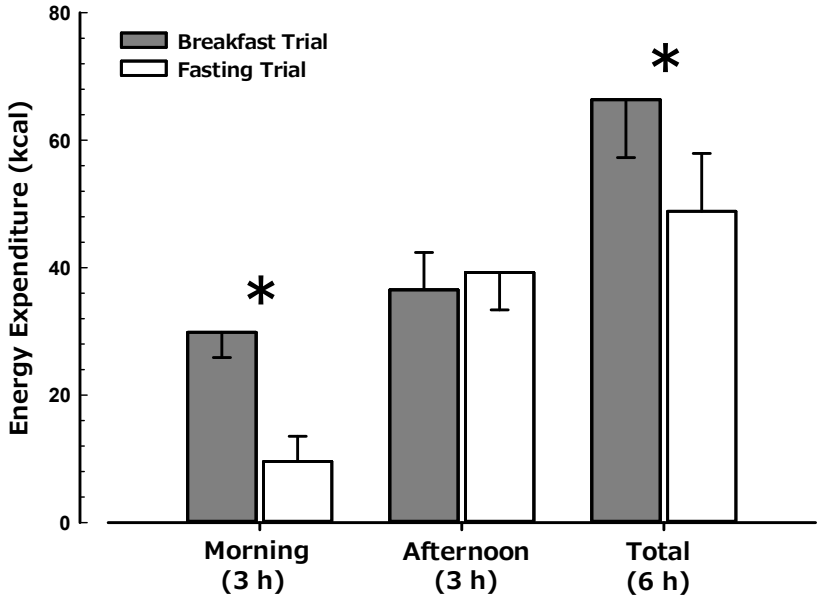

Figure 3.17: Diet induced thermogenesis during trials. The morning and afternoon periods were both of 3 h duration. * $p \leq 0.04$ versus other trial

3.3.11 Diet Induced Thermogenesis

Diet induced thermogenesis during the two trials is displayed in Figure 3.17. Increases in energy expenditure above rest were greater in the morning during the breakfast trial (29.8 ± 13.0 kcal·3h^{-1}) than the fasting trial (9.6 ± 15.2 kcal·3h^{-1}, $p <$ 0.01). After lunch there was no difference between the energy expenditure in either trial (Breakfast Trial, 36.5 ± 22.4 kcal·3h^{-1} *vs* Fasting Trial, 39.3 ± 18.1 kcal·3h^{-1}; $p =$ 0.47). When the morning and afternoon periods were combined; total expenditure was greater ($p = 0.04$) in the breakfast trial (66.4 ± 33.2 kcal·6h^{-1}) than the fasting trial (48.9 ± 28.9 kcal·6h^{-1}).

3.4 Discussion

The aim of the present study was to investigate energy intake at an *ad libitum* lunch meal as well as hormonal, metabolic and subjective appetite responses throughout the day to morning fasting in contrast with a carbohydrate rich breakfast in lean individuals. In this study, energy intake at the *ad libitum* lunch meal was greater following fasting than breakfast consumption. However, this increased intake at lunch was not sufficient to compensate for the intake from the breakfast meal, resulting in a greater net intake in the breakfast trial. However, following lunch consumption, there were contrasting responses from appetite hormones with some indicative of satiety (PYY, Leptin) greater with breakfast but no evidence of ghrelin suppression in the breakfast trial. Subjective appetite three hours after lunch was not different between conditions; indicating that morning fasting does not cause greater appetite in the late afternoon despite limited energy compensation at an *ad libitum* meal.

Despite many experiments that have examined various compositions of breakfast upon subsequent energy intake within a laboratory setting; there have been very few studies in adults investigating the simple comparison of breakfast *vs* morning fasting upon subsequent energy intake at an *ad libitum* lunch. Levitsky and Paconowski (2012) have reported that energy intake at an *ad libitum* lunch was unaffected by breakfasts of ~350 kcal and reduced by ~150 kcal following *ad libitum* breakfast consumption of ~624 kcal. In both cases, the combined energy intake of breakfast and lunch was greater following breakfast consumption in contrast with morning fasting. However, while these feeding occasions were in a laboratory environment, participants were permitted to leave the laboratory between feedings, leading to potential issues with control of extraneous variables and fidelity of the participants to the experimental designs. This work contrasts with Astbury *et al* (2011) who established that increased energy intake at lunch completely compensated for a missed 200 kcal breakfast in male habitual breakfast consumers. However, it has to be considered that these participants were also provided with a 250 kcal pre-load between the breakfast and lunch and therefore the *ad libitum* lunch in the no breakfast trial was not consumed in a fasted state (i.e the responses to lunch are the interactive effects of the breakfast/fasting and the pre-load). Using a similar preload design, Gonzalez *et al* (2013) reported no difference in energy intake at lunch in individuals who either

consumed a 444 kcal breakfast or fasted but were then provided with a 358 kcal preload of chocolate milk 90 minutes prior to the *ad libitum* meal.

As described in Chapter 1, cross sectional evidence is mixed as to whether those that omit breakfast have lower (Cho et al., 2003; Deshmukh-Taskar et al., 2010b; Nicklas et al., 1998) or similar energy intake (Mekary et al., 2013; Mekary et al., 2012; Wyatt et al., 2002). However, this cross sectional data cannot establish cause and effect. In an intervention in which morning meals were prescribed, but delayed by ~3 hours in the no breakfast group and subsequent feeding patterns in the afternoon/evening were structured in both groups, individuals reported lower total daily energy intake during the breakfast intervention (Farshchi et al., 2005b). It would not be realistic to expect in the present study that a ~470 kcal breakfast would be compensated within the one *ad libitum* meal provided. It seems more plausible that the effects of morning fasting may be manifested in greater consumption of snacks and intake at meals later in the day. Future investigations should include greater duration of measurement into the evening and designs that incorporate and quantify the impact of self-initiated feedings. However, emerging evidence in free-living humans suggests that without any dietary restrictions placed upon participants throughout the rest of the day, reported energy intake is similar (Halsey et al., 2012) or greater (Reeves et al., 2014) in those consuming breakfast.

PYY has been shown to reduce food intake (Batterham et al., 2002) with increased caloric load increasing PYY concentrations (Chandarana et al., 2009). In this study, PYY increased in response to breakfast and remained higher than in the fasting trial throughout the afternoon despite increased energy intake at lunch in the fasting trial. This is consistent with the accumulated difference in energy intake over the day and is supported by findings of no difference in PYY concentrations in participants after an *ad libitum* lunch at which participants in the fasting trial ate significantly more, therefore accumulating similar energy intake over the testing day (Astbury et al., 2011). These results therefore indicate that PYY is more a reflection of nutritional status over the entire day rather than in response to the most recent feeding occasion, consistent with PYY peaking 1-2 hours after feeding followed by a lengthy elevation lasting several hours (Adrian et al., 1985).

Leptin concentrations were also higher 3 hours after lunch in the breakfast trial. This finding is consistent with the relatively slow response of leptin to feeding, such that the increased concentrations of leptin ~6 hours after consumption of breakfast are a product of the postprandial responses of glucose and insulin from the breakfast and lunch (Saad et al., 1998b). It would be probable that increases in leptin during the fasting trial may have been delayed and were more likely to occur in the evening, as delaying food intake from 07:00 to 13:30 has previously been shown to concomitantly shift the rise in leptin levels later in the day, further towards the normal late evening zenith (Schoeller et al., 1997).

The increased PYY and leptin concentrations should contribute towards feelings of greater satiety at the end of the trial, although there was no appreciable difference in subjective appetite at the end of the day between the breakfast and fasting trials. This may partly be a product of the complete lack of suppression of ghrelin following lunch in those that had already consumed breakfast. This is a particularly unusual result that to our knowledge has not been reported previously. As it has previously been shown that increasing energy content of liquid breakfasts with increased carbohydrate content causes greater reductions in ghrelin (Blom et al., 2005), it is particularly puzzling that the *ad libitum* meal had no impact upon ghrelin concentrations whatsoever as the lunch intake was high energy/simple carbohydrate. Ghrelin has been associated with meal initiation (Cummings et al., 2001) and as such it would be unexpected for this hormone to be elevated following the second meal of the day. However, work by Foster-Schubert and colleagues (2008) has demonstrated that after feeding with an 80% carbohydrate breakfast drink (providing 20% of daily energy requirements) that ghrelin was initially suppressed but then subsequently rebounded above fasting levels after 3 hours, remaining elevated for the majority of the following 3 hours. An obvious distinction in the present study is that at the point 3 hours after initial feeding the participants received another carbohydrate rich meal. This indicates that participants in the breakfast trial had a completely abolished ghrelin response to subsequent feeding, with ghrelin following the established time course of a similar carbohydrate load without a second feeding occasion.

While not universal (Caixas et al., 2002; Gottero et al., 2003; Schaller et al., 2003), there have been some reports that insulin may play an important role in ghrelin

suppression (Blom et al., 2005; Flanagan et al., 2003; Murdolo et al., 2003; Saad et al., 2002). Therefore, it is interesting to note that after lunch when participants had consumed breakfast, the glucose and insulin responses to the meal over the afternoon were significantly diminished relative to the fasting trial. It is conceivable that the substantial reduction in insulin concentrations in the afternoon following breakfast may have contributed to the absence of ghrelin suppression after lunch. Indeed, this hypothesis is supported by the work of Teff and colleagues (2004) in demonstrating limited suppression of ghrelin by high fructose meals that induced a lesser insulin response than equivalently energetic high glucose meals.

However, this lack of ghrelin suppression needs confirmation and it remains to be seen whether elevated ghrelin concentrations following a lunchtime meal translate to increased hunger and energy intake throughout the rest of the day. It has been previously been suggested in a time-blinded study in men that ghrelin concentrations need to reach a "threshold" (reported as 93% of fasting concentrations) prior to meal requests (Blom et al., 2009). As ghrelin concentrations in the fasting trial had returned to fasting concentrations 3 hours after lunch (the point at which hunger was assessed in the afternoon) this may explain why there was no difference in hunger detected despite greater concentrations of ghrelin in the breakfast trial. Alternatively, ghrelin concentrations following repeated meals may not be as relevant in signalling hunger as prior to the first/second meal of the day.

Whilst the reduction in insulin/glucose concentrations after lunch may be partly explained by the slight reduction in energy intake at lunch following breakfast of ~150 kcal, this is also potentially representative of a second meal effect on glucose (Hamman and Hirschmann, 1919), with an associated reduction of insulin concentration and potentiation of its effects (Bonuccelli et al., 2009). Evidence that this is a "real" reduction in insulin concentrations due to a prior meal and not due to differences in energy intake at lunch is demonstrated in the 11 individuals who ate similar amounts in both trials (either more or <80 kcal less in the breakfast trial relative to the fasting trial). Ten of these individuals had reduced insulin concentrations 60 minutes after lunch during the breakfast trial, with insulin concentrations on average 68 ± 25 % of those in the fasting trial, providing evidence of acutely reduced insulin sensitivity following extended morning fasting.

In a UK population of breakfast consumers, the most frequently (~50% of adults) reported food products consumed during weekdays were cereals and bread/toast (Reeves et al., 2013). This lends some external validity to our choice of breakfast, as although there may be some benefits of higher protein breakfasts (Leidy et al., 2011; Leidy et al., 2013), the majority of the UK population still consume breakfasts that are predominantly rich in carbohydrates.

In summary, while breakfast consumption was incompletely compensated for at an *ad libitum* lunchtime meal, there were increased concentrations of some satiety hormones but no suppression of ghrelin following lunch eaten after breakfast consumption. This may be mediated through reduced insulin concentrations in response to a second meal and result in similar hunger after lunch independent of breakfast consumption/morning fasting. Further work should focus on investigating these responses in obese individuals and using designs that allow greater food choices and self-selected feeding frequency in the afternoon and evening.

Chapter 4: Effect of six weeks of daily morning fasting upon components of energy balance and associated health markers in lean individuals

4.1 Introduction

As discussed in Chapter 1, epidemiology has associated infrequent breakfast consumption with increased risk of adiposity (Ma et al., 2003; Horikawa et al., 2011; Purslow et al., 2008; Barton et al., 2005; Kant et al., 1995), diabetes (Mekary et al., 2013; Mekary et al., 2012) and cardiovascular risk (Cahill et al., 2013b; Smith et al., 2010). However, it is also pertinent that breakfast consumers have also been found to exhibit other healthful behaviours including consumption of less fat and alcohol (van der Heijden et al., 2007), are more likely to be non-smokers (van der Heijden et al., 2007; Smith et al., 2010) and, crucially, are more physically active (Duval et al., 2008). Therefore, despite numerous studies examining associations between breakfast and health, causality cannot be inferred from cross sectional studies. It remains to be established if breakfast is causally linked to health or is instead a marker of a healthy lifestyle.

One of the possible benefits of breakfast consumption is a compensatory reduction in energy intake throughout the rest of the day. However, epidemiological evidence is mixed, with some authors reporting no difference (Song et al., 2005; Wyatt et al., 2002) and others greater intake in those who consume breakfast (Cho et al., 2003; Nicklas et al., 1998). Acute laboratory studies contrasting morning fasting with breakfast have reported both similar (Astbury et al., 2011) and reduced energy intake with the omission of breakfast (Levitsky and Pacanowski, 2013; Gonzalez et al., 2013). Evidence from longer duration randomised controlled trials comparing extended fasting and breakfast consumption is inconsistent, with both greater (Farshchi et al., 2005b), unchanged (Halsey et al., 2012) and lower (Reeves et al., 2014) energy intake when omitting breakfasts in free-living conditions. It is therefore inconclusive whether individuals who extend their morning fast subsequently consume additional calories to compensate throughout the rest of the day.

As well as the potential for reduced energy intake, the other key malleable component of energy balance that may respond to breakfast consumption/morning

fasting is energy expenditure. The studies that have been completed in adults specifically contrasting a daily breakfast regimen with morning fasting have either not measured energy expenditure (Farshchi et al., 2005b; Schlundt et al., 1992) or have used partial records of physical activity assessed via pedometers and heart rate monitoring (Halsey et al., 2012), which limits the sensitivity of the physical activity measurement undertaken (Butte et al., 2012). Halsey and colleagues (2012) report when individuals skipped or consumed breakfast for a week, there were no differences in physical activity between conditions, but as well as issues with their choice of measurement device, the choice of monitoring period also limits the validity of their observations. Physical activity as assessed by these instruments was only measured during the hours between 09:00 and 17:00 in campus based university students. Therefore, the levels of physical activity observed will be predominantly dictated by the schedule of activities associated with studying.

Results from a previous randomised controlled trial examining 2 weeks of extended fasting in comparison with breakfast consumption in lean women has revealed a negative impact of the extended fasting intervention upon fasting lipids and insulin sensitivity (Farshchi et al., 2005b). These findings agree with several prospective studies implicating omission of breakfast with greater incidence of diabetes (Mekary et al., 2013; Mekary et al., 2012), coronary heart disease events (Cahill et al., 2013b) and development of overall metabolic risk (Odegaard et al., 2013). The work of Farshchi and colleagues (2005) was of a relatively short duration with experimenter prescription of feeding frequency and food items consumed. It remains to be established if a less externally regulated intervention manipulating morning energy intake has similar effects.

In studies that have compared breakfast consumption with extended fasting, all have involved experimenter prescription of either feeding frequency (Farshchi et al., 2005b), or breakfast composition (Halsey et al., 2012) or a combination of both factors with a target energy deficit (Schlundt et al., 1992), or weight stability (Stote et al., 2007). In a less prescriptive study, only diet was examined in response to an extended morning fasting intervention (Reeves et al., 2014). A randomised study examining the “natural” responses of free-living individuals to a morning fasting

intervention that has quantified all components of energy balance is yet to be conducted.

The aim of the current study is to examine the impact of daily morning fasting/breakfast interventions on energy balance and selected markers of health in lean individuals. On the basis of previous investigations we hypothesise that energy intake will be lower in individuals fasting during the morning but that blood lipids and insulin sensitivity will be negatively affected by daily morning fasting.

4.2 Participant and Methods

4.2.1 Participants

Thirty-three healthy, lean men (n = 12) and women (n = 21) aged 22-56 y volunteered to take part in this study. Participants were deemed suitable for participation based upon full eligibility criteria outlined in Chapter 2. Participants were recruited via local advertisement from South West England and were initially assessed for eligibility based upon a body mass index of 18-25 $kg \cdot m^{-2}$ and then later classified as lean based upon DEXA-derived fat mass indices of ≤7.5 $kg \cdot m^{-2}$ (men) and ≤11 $kg \cdot m^{-2}$ (women) (Kelly et al., 2009). Participants reported being weight stable (± 2% body mass within past 6 months) and adhered to a standard sleep-wake cycle (e.g no shift workers) and did not anticipate any change in lifestyle during the study period. Participants were free of metabolic disorders, with pre-menopausal female participants either menstruating regularly, or following their chosen contraceptive method (i.e pill, implant) for >6 months. These individuals were randomly assigned to one of two intervention groups; either a group prescribed 700 $kcal \cdot d^{-1}$ before 11:00 with at least half of intake within 2 hours of waking or a group that extended their overnight fast until 12:00 daily for 6 weeks. Characteristics of the two experimental groups are presented in Table 4.1.

Written informed consent was obtained from all participants, with the Ethical Approval for the study obtained from the NHS Bristol Research Ethics Committee. The study is registered with Current Controlled Trials [ISRCTN31521726].

Table 4.1: Participant characteristics

Characteristic		Breakfast Group	Fasting Group
n		16	17
Age (y)		36 (11)	36 (11)
Body Mass (kg)		67.0 (8.3)	66.5 (7.8)
Body Mass Index (kg/m^2)		22.0 (2.2)	22.8 (2.3)
Fat Mass Index (kg/m^2)*	**All**	5.4 (2.2)	5.9 (2.3)
	Female	6.5 (2.1)	6.8 (2.0)
	Male	3.6 (1.0)	4.1 (1.6)
Habitual Breakfast Consumers (*n*)		11	15
Female (*n*)		10	11

*Fat mass index calculated as DEXA derived total fat mass divided by height squared. Data presented are Mean with (SD)

4.2.2 Study Design

The study employed an independent measures comparison of 2 randomly assigned parallel groups. Participants were randomised using a block randomisation plan, with stratification to facilitate an equal distribution of habitual consumers (defined as the ingestion of ≥50 kcal·d^{-1} within two hours of waking on most days of the week) and non-consumers in the two groups. Following two days of diet and activity standardisation both groups had baseline measures of body composition and insulin sensitivity (OGTT) assessed. Subsequently, the breakfast group were prescribed 700 kcal·d^{-1} before 11:00 (with at least half within two hours of waking) whilst the fasting group extended their overnight fast until 12:00 daily for 6 weeks. During the first and last week of the intervention participants had measures of physical activity energy expenditure, energy intake and glucose variability assessed under free-living conditions. Following repetition of the standardisation procedures, participants had body composition and insulin sensitivity assessed again upon completion of the 6 week intervention. A schematic representation of the experimental design is provided in Figure 4.1.

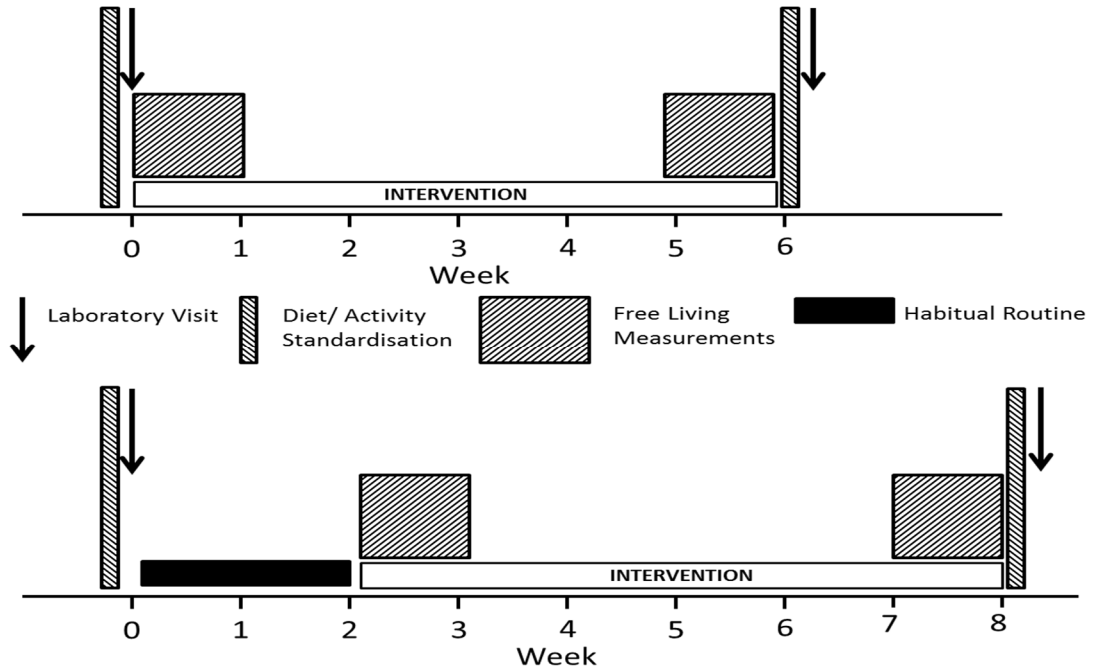

Figure 4.1: Schematic representation of study design. The top half represents the normal schedule, the bottom half for a menstruating female.

4.2.3 Prescribed Breakfast

As the first study to have quantified all elements of energy balance, the two interventions that the groups undertook were designed to maximise the discrepancy between the experimental conditions. As such, the fasting group was to abstain from energy until 12:00 each day and those participants that were allocated to the breakfast consumption group were required to consume ≥700 kcal by 11:00 daily as part of their intervention period. This quantity of breakfast was selected as it was deemed a realistic energetic target as a breakfast of this size has been employed previously in a free-living intervention (Martin et al., 2000). In addition, this energy content fits within the previously suggested theoretical energy content of what constitutes a breakfast (i.e 20-35% of daily EI) of Timlin and Pereira (2007). Participants were allowed to consume self-selected foods to reach this energy content. In order to assist this process, participants were provided with an example booklet of common foodstuffs and their energy content such that they could estimate the energy content of foods consumed.

4.2.4 Laboratory Experimental Protocol

Participants reported to the laboratory overnight fasted and having followed standardisation procedures as described in Chapter 2. Participants were asked to void and were subsequently weighed in light clothing (Body Composition Monitor BC-543, Tanita, Japan) and underwent a DEXA scan. Participants were then fitted with an indwelling cannula in an antecubital vein with a 10 mL resting venous sample obtained. A small (~0.5 g) subcutaneous adipose tissue biopsy was then obtained under local anaesthetic. Following a period of applied pressure to the biopsy site (~10 min), participants received a 75 g oral glucose load in a 300 mL total volume. Commencing upon complete ingestion of the fluid volume, 5 mL blood samples were obtained at 15 minute intervals with the final sample at 120 minutes post-ingestion. Full details of all measurements and analyses are provided in Chapter 2.

4.2.5 Free-Living Experimental Protocol

On the day prior to commencement of the assigned 6 week free-living intervention (which commenced immediately after the baseline laboratory measures in men and non-menstruating women but was delayed by two weeks in menstruating women to ensure follow-up testing occurred in the same point in the menstrual cycle;

Figure 4.1) participants were fitted with a continuous glucose monitor (iPro®), a physical activity monitor (Actiheart®) and provided with a food/activity record and scales. Participants then wore these measurement devices for a continuous period of 7 days excluding the day of fitting, while concurrently recording a weighed food intake and completing a physical activity diary.

At this point it was emphasised to participants that the design of the experiment was not to place any other restrictions upon any element of their lifestyle other than the consumption/omission of breakfast. As such their recording diary during this period contained the following text.

"Your lifestyle choices during this free-living monitoring period are central to this study. We are interested in any natural changes in your diet and/or physical activity habits, which you may or may not make in response to the intervention. This monitoring period has been carefully scheduled to avoid any pre-planned changes in these habits, such as a holiday or diet/exercise plan. You should inform us immediately if unforeseen factors external to the study may influence your lifestyle."

Following this initial observation period, participants continued with their assigned intervention with no further measurements until ten days prior to completion of their 6 week intervention. At that point, participants were fitted with the same measurement devices as week 1, with the same duration of 7 days of continuous measurement. This final period of measurement was followed by two days of exact replication of the standardisation practices that preceded each participant's initial laboratory visit to assess baseline laboratory measures. Following these two days of standardisation participants then repeated the laboratory protocol outlined previously. Further details on the methods used are provided in Chapter 2.

4.2.6 Statistical Analysis

For repeated measurements in both groups (i.e Physical activity energy expenditure, energy intake) 2-way mixed model ANOVA were employed. For comparisons of means, either paired or independent measures t-testing was conducted as appropriate. Data are presented in text as means with standard deviations, in tables

change scores are presented with 95 % confidence intervals and error bars displayed on graphs are standard error of the mean. Statistical significance was accepted at $p \leq 0.05$, with all analyses conducted using IBM SPSS statistics version 22 (IBM, New York).

4.3 Results

4.3.1 Body Composition

There was no significant difference in mass between the fasting and breakfast groups at baseline (66.5 ± 7.8 kg vs 67.0 ± 8.3 kg, respectively; F = 0.05, p = 0.8). While there was a trend towards reduced mass in both groups from pre- to post-intervention (F = 3.602, p = 0.07), the pre-planned contrast for change in mass within each group revealed significant weight-loss in the fasting group only (0.43 ± 0.73 kg; p = 0.03) Weight change was more variable across individuals in the breakfast group, resulting in no significant changes in body mass over time (0.19 ± 1.16 kg; p = 0.5).

Table 4.2: Anthropometric measures

Measure	*Breakfast*		*Fasting*	
	Pre	Change	Pre	Change
Total Mass (kg)	67.0 (8.3)	-0.2 (-0.8, 0.4)	66.5 (7.8)	-0.4 (-0.8, -0.1)
Lean Mass (kg)	47.7 (9.4)	-0.08 (-0.72, 0.57)	46.4 (8.1)	-0.08 (-0.65, 0.49)
Fat Mass (kg)	16.2 (5.7)	-0.21 (-0.76, 0.34)	16.9 (6.2)	-0.21 (-0.91, 0.49)
Waist:Hip ratio	0.77 (0.06)	-0.01 (-0.02, 0.00)	0.80 (0.07)	-0.01 (-0.02, 0.00)
Sagittal Abdominal Diameter (cm)	18.3 (1.7)	-0.5 (-1.0, -0.0)	18.6 (1.4)	-0.5 (-0.9, -0.0)

Values represent mean with (SD) and change scores from baseline with (95 % CI)

There were no significant differences between the groups for either fat or lean mass (p > 0.6) and neither did fat (F = 0.970, p = 0.3) nor lean mass (F = 0.144, p = 0.7) vary significantly with respect to time. Measures of central adiposity were not different between groups and did not respond differently to the intervention for any measure between groups (all p > 0.2). Across both groups, sagittal abdominal diameter decreased as a result of the intervention (both p < 0.01).

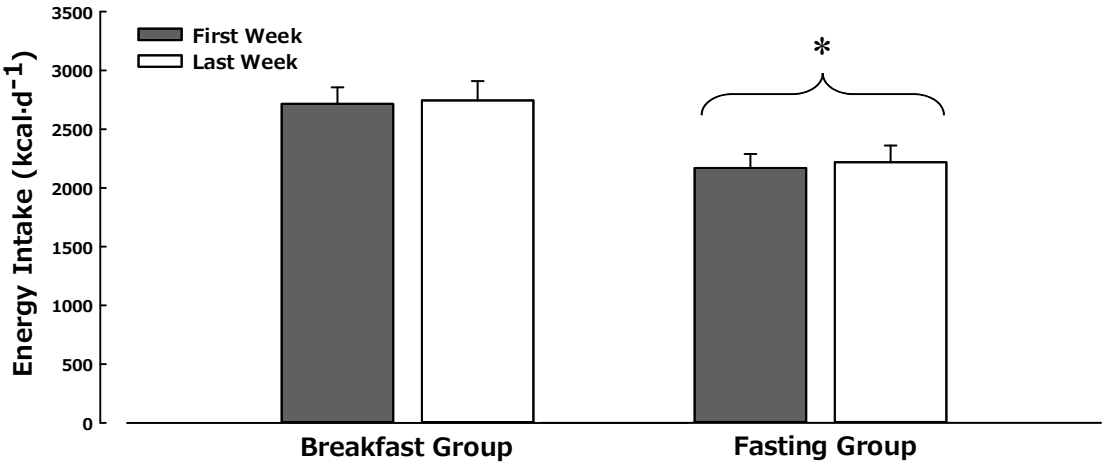

Figure 4.2: Reported energy intake during intervention. *lower daily energy intake in fasting than breakfast group ($p < 0.01$)

4.3.2 Energy Intake

The fasting group had a significantly lower reported mean energy intake during the measurement period relative to the breakfast group (2191 ± 494 kcal·d^{-1} *vs* 2730 ± 573 kcal·d^{-1}; $p < 0.01$). Energy intake was stable (within 50 kcal·d^{-1}) between the first and last week of the intervention.

Comparisons of percentage macronutrient composition of diets are provided in Table 4.3. Analysis of relative macronutrient content for the whole day between the two groups indicated no main effects of time or group, apart from a lower relative protein intake in the breakfast group ($F = 5.152$, $p = 0.03$). The percentage protein intake was 15.6 ± 3.3 % and 15.6 ± 2.7 % for the fasting group, and 14.1 ± 2.0 % and 13.5 ± 1.7 % for the breakfast group in the first and last weeks, respectively.

When comparing equivalent time periods during which both groups had unrestricted access to foods (i.e 12:00 onwards); there was a strong tendency for the mean energy intake of both weeks to be greater in those fasting (2193 kcal·d^{-1} *vs*. 1852 kcal·d^{-1}; $p = 0.06$). There were no differences between the post 12:00 relative carbohydrate or fat intake, either between groups or with respect to time (all $p > 0.7$). Relative protein intake was not different between groups (Fasting Group, 15.6 ± 2.8 % *vs* Breakfast Group, 14.2 ± 1.7 %; $p = 0.09$).

In the breakfast group there were no differences between the pre 12:00 total energy intakes for the first and last week of the intervention (867 ± 110 kcal·d^{-1} *vs.* 880 ± 149 kcal·d^{-1}) or any differences in macronutrient intake between the two weeks of monitoring. When comparing the pre vs post 12:00 relative macronutrient composition of those consuming breakfast, relative carbohydrate intake was significantly higher in the morning than after 12:00 (57.3 ± 9.5 % *vs* 45.0 ± 7.3 %; $p < 0.01$). There was a strong tendency for fat intake to be lower in the morning (29.8 ± 8.2 *vs* 34.6 ± 5.4 %, $p = 0.06$), but relative protein intake was not significantly different ($p = 0.1$).

Table 4.3: Percentage composition of diets during intervention

	Breakfast Group						**Fasting Group**	
	First Week			**Last Week**			**First Week**	**Last Week**
	Pre 12:00	**Post 12:00**	**Total**	**Pre 12:00**	**Post 12:00**	**Total**	**Post 12:00**	**Post 12:00**
Energy Intake (kcal·d^{-1})	867 (112)	1849 (487)	2715 (565)	889 (147)	1856 (564)	2745 (658)	2169 (490)*	2218 (587)*
Protein %	13.2 (2.9)	14.5 (2.0)	14.2 (2.1)	12.7 (2.4)	13.9 (1.9)	13.5 (1.7)	15.6 (3.3)#	15.6 (2.7)#
Carbohydrate %	57.9 (9.0)†	45.3 (7.3)	48.5 (6.2)	56.7 (10.9)†	44.7 (8.0)	47.7 (7.2)	45.5 (6.4)	46.4 (6.8)
Fat %	29.0 (8.3)	34.5 (5.5)	33.5 (4.6)	30.6 (9.4)	34.8 (5.6)	34.5 (4.5)	34.4 (4.6)	34.3 (4.3)
Alcohol %	n/a	5.7 (6.1)	3.8 (4.6)	n/a	6.6 (6.4)	4.4 (4.0)	4.5 (3.7)	3.5 (3.5)

Values represent mean with (SD)
* Denotes main effect of group ($p < 0.01$), with reduced energy intake in fasting group.
# Denotes main effect of group ($p = 0.03$), with higher relative intake in fasting group.
† Denotes significantly higher ($p < 0.01$) pre 12:00 versus post 12:00 in breakfast group.

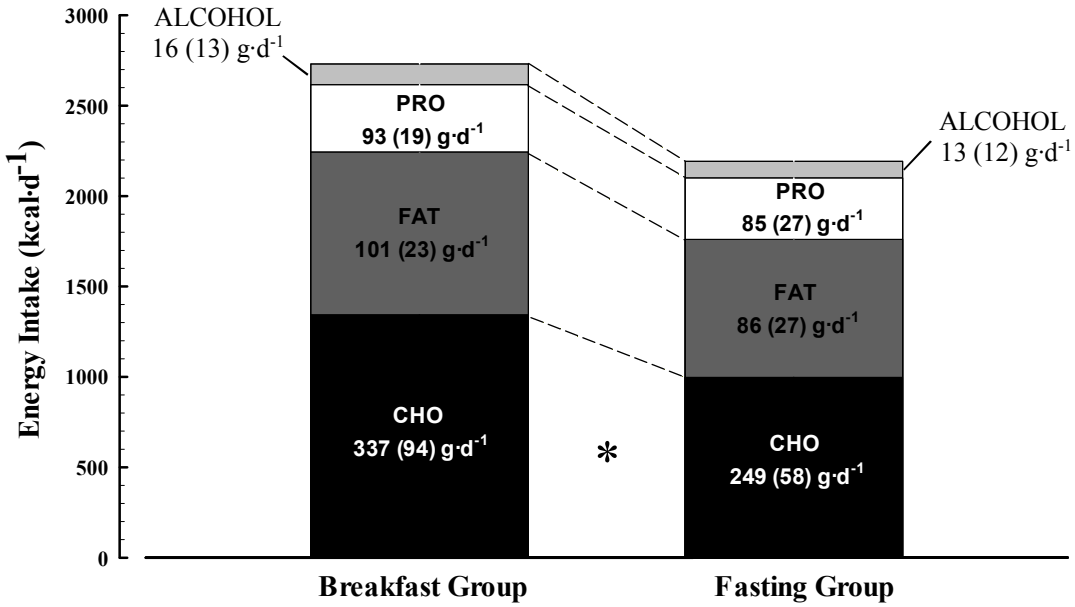

Figure 4.3: Daily macronutrient intake during the intervention *significantly lower carbohydrate intake in fasting than breakfast group ($p < 0.01$). Figures on stack represent mean intake, with figures in brackets representing standard deviation.

Comparisons of the mean daily macronutrient intakes during the intervention indicated that carbohydrate intake was lower in the fasting group ($p < 0.01$, Figure 4.3). There was a tendency for lower fat intake with fasting ($p = 0.08$), but protein and alcohol intake was not different between groups (both $p > 0.3$). Total sugar intake was lower in the fasting group than the breakfast group (96 ± 38 g·d^{-1} *vs.* 149 ± 51 g·d^{-1}; $p < 0.01$) as was saturated fat intake (29 ± 11 g·d^{-1} *vs.* 37 ± 11 g·d^{-1}; $p = 0.03$).

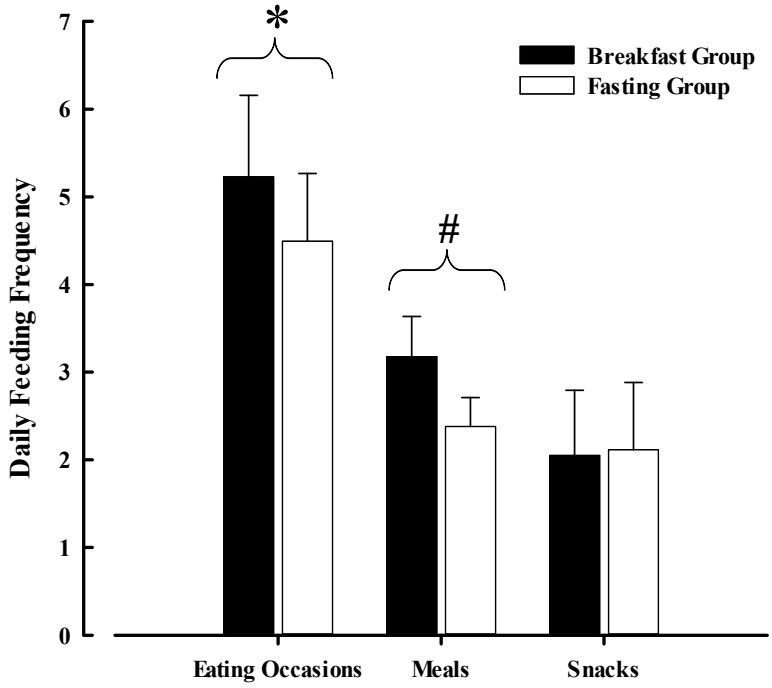

Figure 4.4: Daily feeding frequency, *fewer eating occasions in fasting group (p = 0.03) # fewer meals in fasting group ($p < 0.001$)

4.3.3 Feeding Frequency

Analysis of daily feeding frequency showed that total eating occasions were greater in the breakfast group relative to the fasting group (5.22 ± 0.93 *vs.*4.49 ± 0.77; p = 0.03). This was primarily driven by the increased daily meal frequency (3.18 ± 0.46 *vs.* 2.38 ± 0.33; $p < 0.01$), consistent with and directly proportional to the breakfast that was prescribed/omitted in each groups. In contrast, daily snacking frequency was similar in the breakfast (2.05 ± 0.74) and fasting groups (2.11 ± 0.77; p = 0.8).

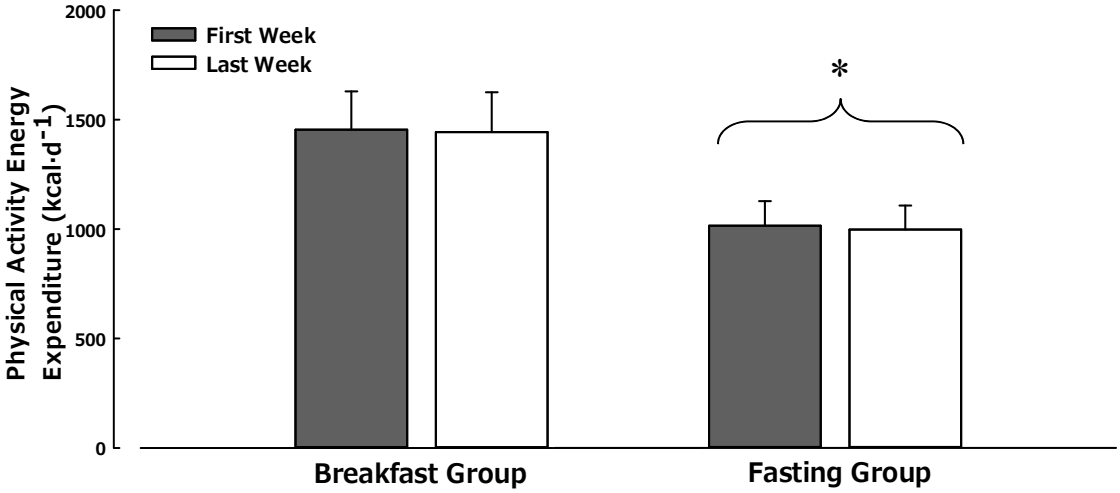

Figure 4.5: Physical Activity Energy Expenditure *lower in fasting than breakfast group ($p = 0.03$).

4.3.4 Physical Activity Energy Expenditure

The mean physical activity energy expenditure averaged over the intervention was significantly lower in the fasting group (1007 ± 370 kcal·d^{-1}) relative to those in the breakfast group (1449 ± 666 kcal·d^{-1}; F = 5.054, $p = 0.03$). There was ≤ 50 kcal·d^{-1} difference between the PAEE for the first and last week of the intervention in both groups.

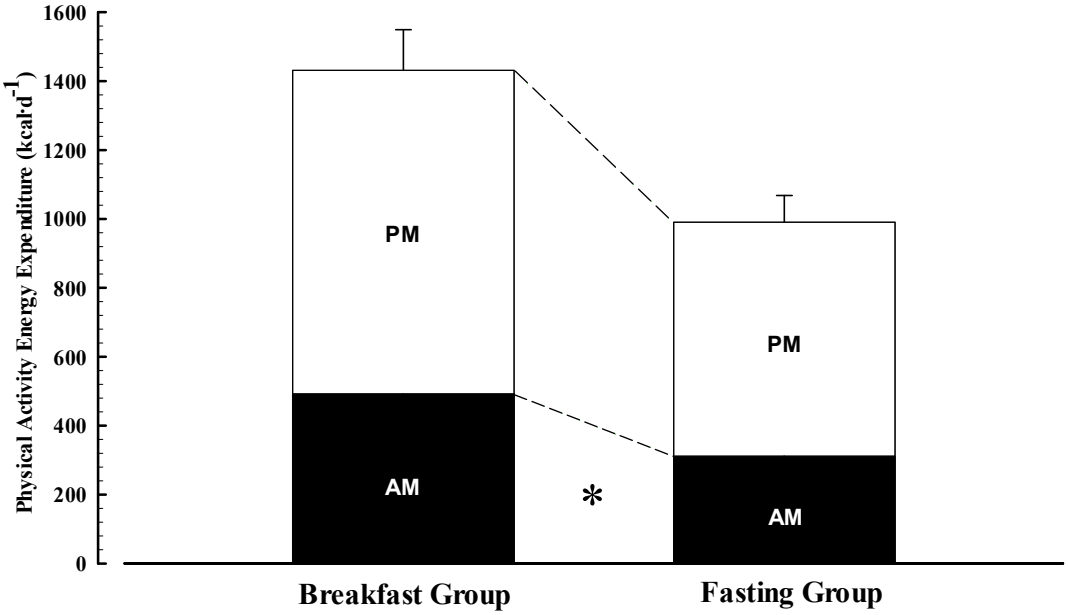

Figure 4.6: AM/PM split of physical activity energy expenditure *lower in fasting than breakfast group ($p = 0.01$).

The data for energy expenditure split by time of day are presented in Figure 4.6 and Table 4.4. Total energy expenditure in the AM period was significantly lower in the fasting group (311 ± 124 kcal·d^{-1} *vs.* 492 ± 227 kcal·d^{-1}; $p = 0.01$). There was also a trend ($p = 0.08$) towards lower energy expenditure in the PM period in those fasting (680 ± 301 kcal·d^{-1}) compared with those consuming breakfast (938 ± 457 kcal·d^{-1}).

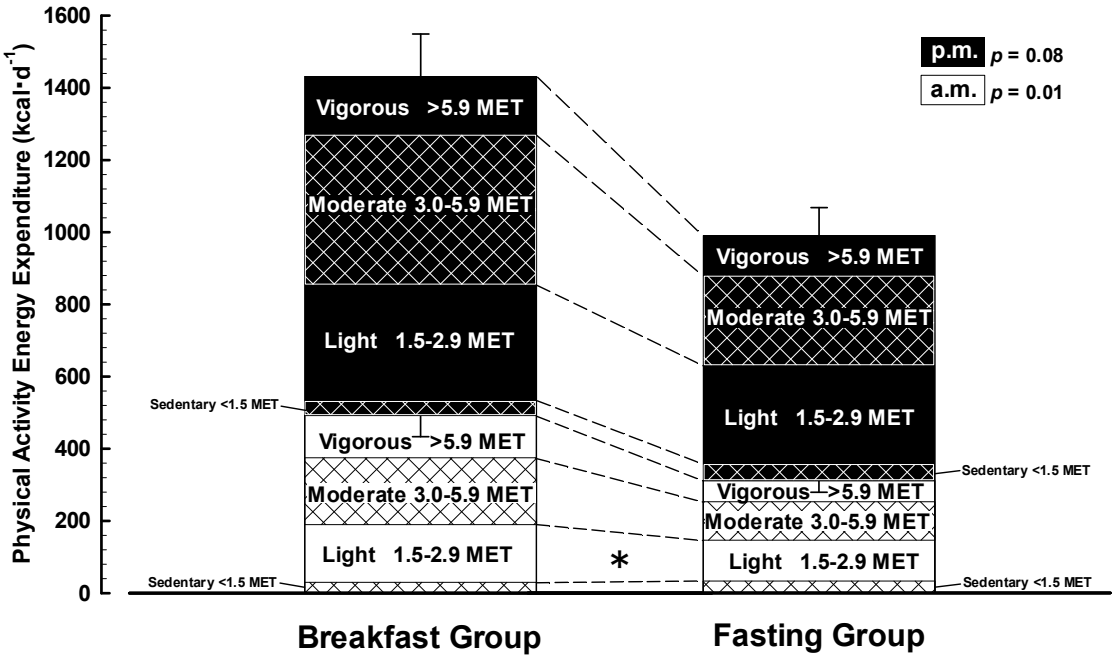

Figure 4.7: Physical activity energy expenditure split by time of day and intensity
* significantly lower in fasting than breakfast group ($p = 0.03$)

Data for energy expenditure split both by time of day and intensity thresholds are presented in Figure 4.7. Energy expenditure classified as of light intensity was significantly lower in those fasting during the AM period than those consuming breakfast (114 ± 41 kcal·d^{-1} *vs.* 161 ± 41 kcal·d^{-1}; $p = 0.03$).

Table 4.4: Physical activity energy expenditure split by intensity and time of day

	Breakfast Group			Fasting Group		
Energy Expenditure (kcal·d^{-1})	**AM**	**PM**	**24h**	**AM**	**PM**	**24h**
Sedentary (<1.5 MET)	27 (12)	40 (12)	67 (22)	32 (20)	48 (22)	80 (40)
Light (1.5-2.9 MET)	161 (41)	324 (99)	486 (133)	114 (41)*	274 (96)	387 (131)
Moderate (3.0-5.9 MET)	182 (111)	411 (320)	593 (423)	109 (61)	245 (134)	353 (186)
Vigorous (6.0-10.1 MET)	76 (59)	95 (60)	171 (103)	33 (22)	57 (72)	90 (88)
Very Vigorous (≥10.2 MET)	46 (61)	68 (83)	114 (131)	24 (47)	57 (109)	80 (155)
Total	492 (227)	938 (457)	1431 (657)	311 (124)*	680 (301)	990 (415)*

Values represent mean with (SD)
* Denotes significantly lower in fasting than breakfast group ($p < 0.05$)

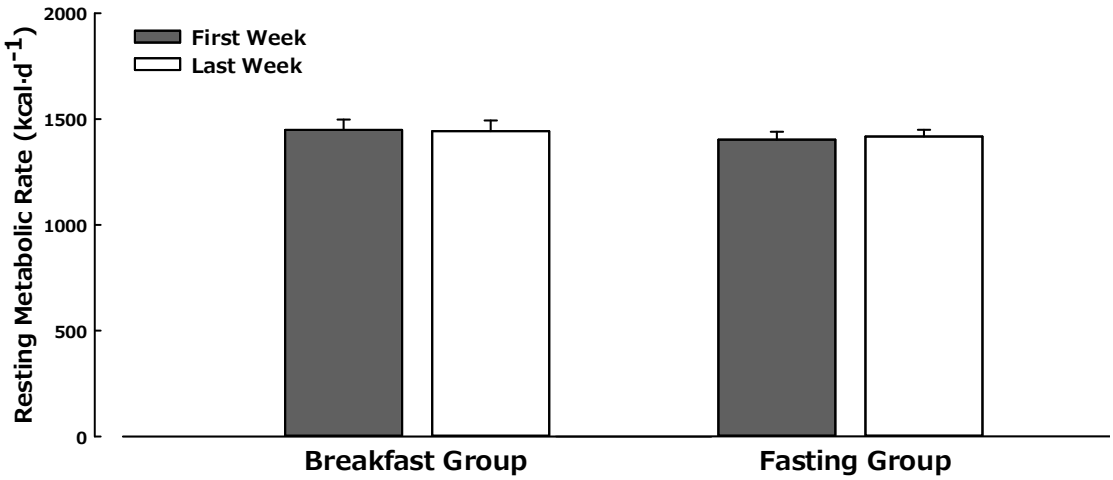

Figure 4.8: Resting metabolic rate before and after the intervention.

4.3.5 Resting Metabolic Rate

Both the groups had similar resting metabolic rates prior to the intervention (Breakfast Group, 1449 ± 195 kcal·d^{-1} *vs.* Fasting Group, 1402 ± 149 kcal·d^{-1}, Figure 4.8). These values were stable in response to the intervention with a <15 kcal·d^{-1} difference from pre- to post-intervention and no difference in response between groups (Breakfast Group, 1443 ± 201 kcal·d^{-1} *vs.* Fasting Group, 1417 ± 129 kcal·d^{-1}; all $p >$ 0.5).

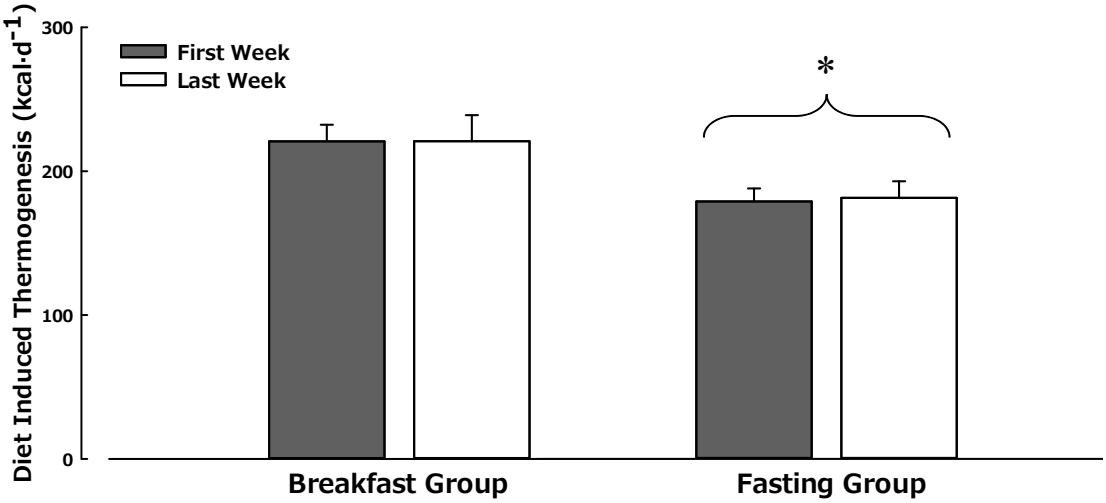

Figure 4.9. Diet induced thermogenesis during the intervention, * significantly lower in fasting than breakfast group ($p < 0.05$)

4.3.6 Diet Induced Thermogenesis

Based on the established thermogenic effect of ingested macronutrients, diet induced thermogenesis was lower in the fasting group than in the breakfast group (180 ± 39 kcal·d^{-1} *vs*. 221 ± 49 kcal·d^{-1}; $p < 0.05$, Figure 4.9). The group difference in energy intake of 539 kcal·d^{-1} equated to a difference in DIT of 41 kcal·d^{-1}. There was less than a 5 kcal·d^{-1} difference from the first to last week of the intervention within groups.

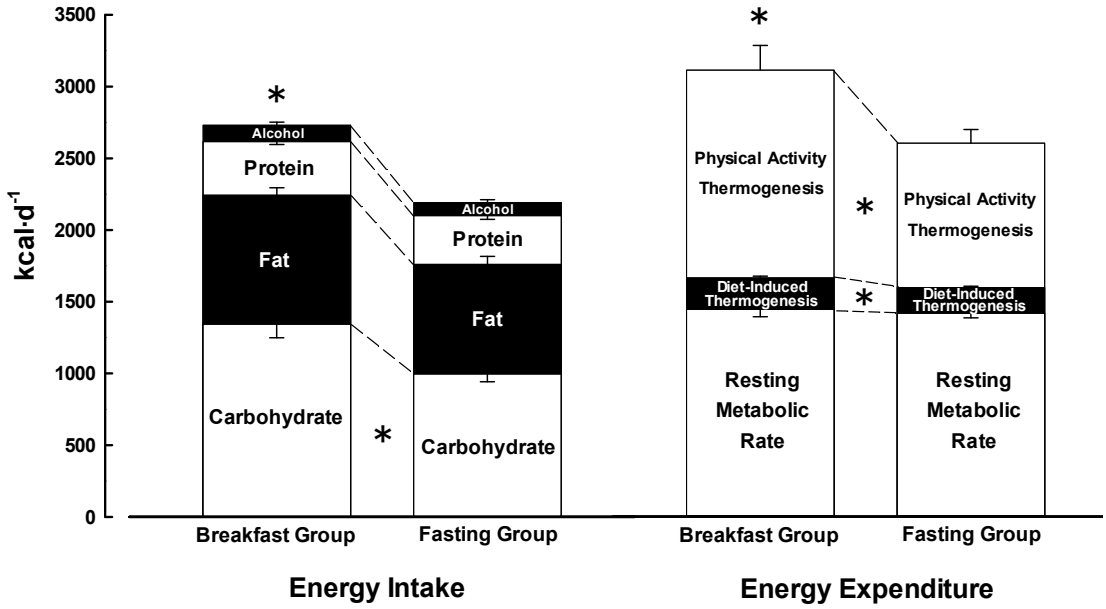

Figure 4.10: Energy Balance Summary. An asterisk above a bar represents the comparison between the sum of the bars, an asterisk between the bars represents the comparison between the specific component. * $p < 0.05$ for difference between groups.

4.3.7 Oral Glucose Tolerance Test

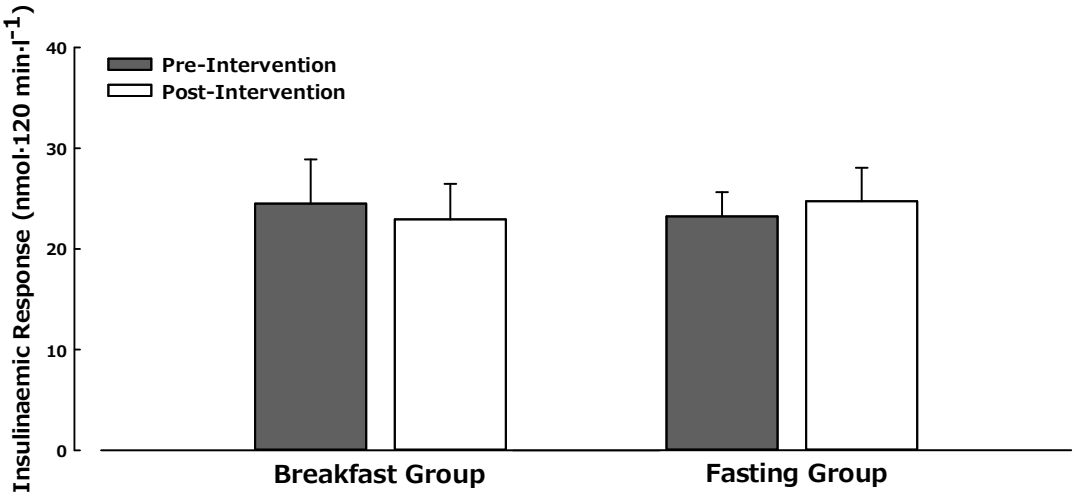

Figure 4.11: Insulinaemic response to an Oral Glucose Tolerance Test

There were no significant differences either between groups (F = 0.00, p = 0.9), from pre-post (F = 0.0, p = 0.9) or an interaction effect (F = 0.86, p = 0.36) upon the incremental area under the curve calculated for the insulinaemic response to the oral glucose tolerance test.

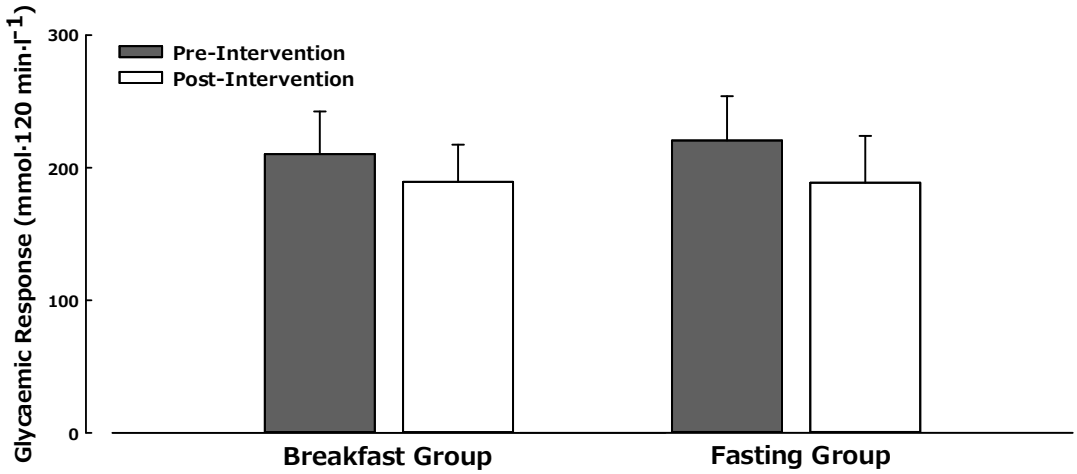

Figure 4.12: Glycaemic response to an Oral Glucose Tolerance Test

There was no difference between the groups (F = 0.01, p = 0.9) or in response to the intervention (F = 2.2, p = 0.15) for the glycaemic response to an oral glucose tolerance test. The fasting and breakfast groups did not differ in their response to the intervention (F = 0.09, p = 0.7).

Table 4.5: Insulin sensitivity measures

Measure	*Breakfast Group*		*Fasting Group*	
	Pre	Change	Pre	Change
Fasting Insulin ($pmol.l^{-1}$)	20.2 (12.9)	2.1 (-1.9, 6.0)	20.6 (8.6)	1.9 (-4.0, 7.8)
Peak Insulin ($pmol.l^{-1}$)	364 (210)	37 (-62, 136)	357 (182)	36 (-14, 87)
Fasting Glucose ($mmol.l^{-1}$)	5.25 (0.31)	0.06 (-0.15, 0.27)	5.36 (0.31)	0.07 (-0.10, 0.24)
Peak Glucose ($mmol.l^{-1}$)	8.73 (1.27)	-0.2 (-1.02, 0.60)	9.00 (1.57)	-0.04 (-0.92, 0.83)
HOMA-IR	0.79 (0.52)	0.10 (-0.06, 0.26)	0.83 (0.36)	0.10 (-0.16, 0.36)
C-ISI	12.1 (6.6)	-1.0 (-3.7, 1.8)	11.1 (8.0)	0.4 (-1.3, 2.1)

Values represent mean with (SD) and change scores from baseline with (95 % CI)

4.3.8 Insulin Sensitivity Measures

A summary of the measures obtained from the OGTT's are presented in Table 4.5. There were no effects of group ($p > 0.9$), or any interaction effects ($p > 0.9$) for either peak or fasting insulin. Neither measure was different following the intervention ($p > 0.14$). For both peak and fasting glucose there were no significant effects of group, time or any interaction effects (all $p > 0.3$). Additionally, there were no main effects ($p > 0.2$) or interactions ($p > 0.3$) for either HOMA-IR or the C-ISI indices of insulin sensitivity.

Table 4.6: Fasting lipids and inflammatory markers

Measure	***Breakfast Group***		***Fasting Group***	
	Pre	Change	Pre	Change
Total Cholesterol ($mmol.l^{-1}$)	4.98 (1.15)	0.13 (-0.23, 0.48)	5.00 (0.59)	0.22 (-0.06, 0.50)
HDL Cholesterol ($mmol.l^{-1}$)	1.37 (0.32)	0.12 (0.03, 0.22)	1.28 (0.27)	0.11 (0.05, 0.18)
LDL Cholesterol ($mmol.l^{-1}$)	3.22 (0.92)	0.01 (-0.25, 0.27)	3.34 (0.64)	0.14 (-0.08, 0.35)
Total:HDL Cholesterol	3.71(0.79)	-0.21 (-0.39, -0.04)	4.07 (1.02)	-0.18 (-0.37, 0.02)
Triglycerides ($mmol.l^{-1}$)	0.86 (0.37)	-0.01 (-0.14, 0.11)	0.84 (0.19)	-0.06 (-0.18, 0.06)
NEFA ($mmol.l^{-1}$)	0.55 (0.19)	0.06 (-0.08, 0.19)	0.61 (0.30)	-0.07 (-0.22, 0.07)
CRP ($mg.l^{-1}$)	0.79 (0.82)	-0.13 (-0.46, 0.21)	0.53 (0.50)	-0.10 (-0.36, 0.16)

Values represent mean with (SD) and change scores from baseline with (95 % CI)

4.3.9 Cardiovascular Disease Risk Factors

There were no significant differences between any of the fasting lipids or CRP concentrations measured between the groups (all $p > 0.2$). For all of the parameters measured there was no evidence of any interaction effects in response to the intervention (all $p > 0.17$). HDL cholesterol concentration was significantly increased across both groups from pre- to post-intervention ($F = 17.22$, $p < 0.01$) (Table 4.6). The Total:HDL cholesterol ratio was significantly reduced across both groups as a result of the interventions ($F = 9.99$, $p < 0.01$). None of the other lipids or inflammatory markers measured responded to the intervention over time (all $p > 0.1$).

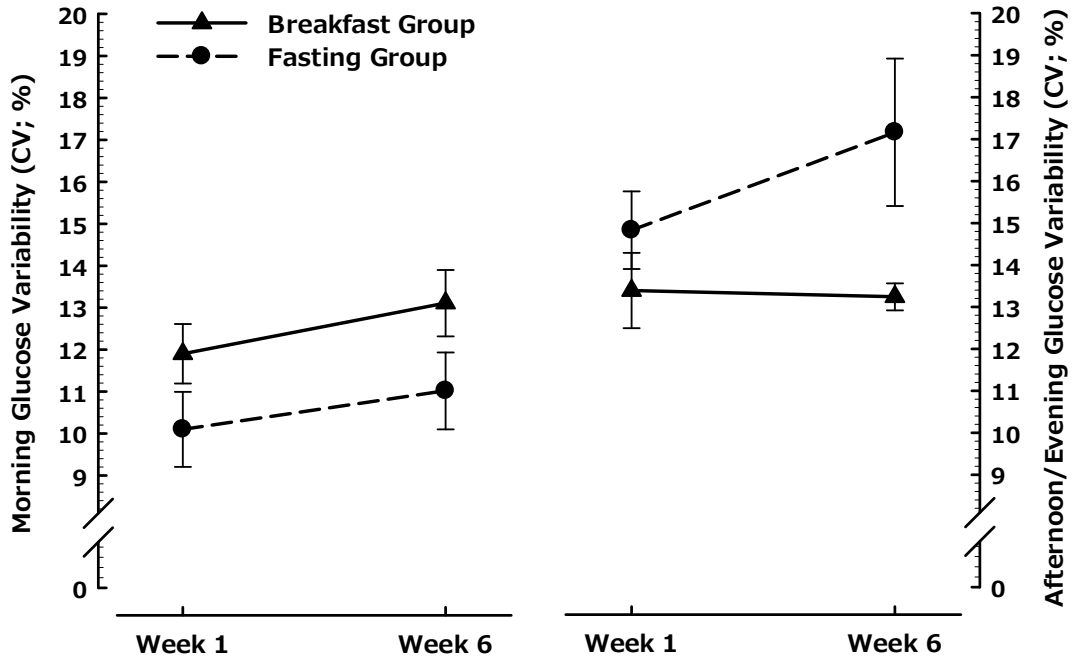

Figure 4.13: Glucose variability from waking until 12:00 (left hand side) and from 12:00 until sleeping (right hand side) during the first and last week of the intervention.

4.3.10 Continuous Glucose Monitoring

Continuously measured glucose indicated that from waking until 12:00 the mean (5.4 ± 0.5 mmol.l^{-1} *vs* 5.1 ± 0.5 mmol.l^{-1}) and peak (7.6 ± 1.2 mmol.l^{-1} *vs* 6.5 ± 1.0 mmol.l^{-1}) glucose concentrations were greater ($p < 0.04$) in the breakfast group, with no effects of time or interaction effects ($p \geq 0.1$). The coefficient of variation (CV) for glucose indicated greater glucose variability in the morning in the breakfast group ($F = 4.90$ $p = 0.04$; Figure 4.13). The fasting group exhibited greater glucose variability from 12:00 to sleeping across both weeks ($F = 4.29$, $p = 0.05$). The greatest difference between the groups was during week 6 (3.9%, 95% CI: 0.1%, 7.8%). Other results indicated there were no main effects or interactions (all $p > 0.1$) for peak, mean or glucose variability during the morning, from 12:00 until sleep, during sleep and over the full 24 hour period (see Appendix 3).

4.4 Discussion

The current study has examined all elements of energy balance, as well as metabolic control and markers of cardiovascular disease risk in response to both daily morning fasting and breakfast consumption for 6 weeks in healthy lean individuals. Energy intake was significantly lower in those that extended their fast until 12:00, with limited compensation for the omission of intake in the morning relative to the breakfast consumption group. However, daily physical activity energy expenditure was lower in those that extended their fast through the morning, which may cause negative health implications. Markers of metabolic control and cardiovascular health were mostly unaffected by either intervention, although there was tentative evidence that daily fasting may result in increasing glucose variability during the afternoon and evening. Contrary to cross-sectional evidence, daily morning fasting did not cause increased weight.

The present data indicate significantly lower energy intake in free living individuals who fasted until 12:00 each day relative to those with a prescribed 700 ≥ $kcal \cdot d^{-1}$ energy intake by 11:00 but otherwise completely self-selected meal composition and feeding frequency. This is in contrast to the previous work of Farshchi and colleagues (2005) who report greater total energy intake in those omitting breakfast relative to those consuming breakfast. However, in the aforementioned study during the omission condition participants actually consumed the same "breakfast" in two stages as during the breakfast consumption condition but ~2 hours later. In addition, feeding frequency was then fixed throughout the remainder of the day. In contrast, two studies with less prescription of diet outside the morning period have resulted in similar (Halsey et al., 2012) and lower (Reeves et al., 2014) daily energy intakes in those fasting during the morning.

Many studies within the literature utilise an acute laboratory-based experimental paradigm to investigate feeding behaviours, with elements of this approach also incorporated into this work. This allows accurate characterisation of physiological parameters and tight control of external influences. However, the key difference between laboratory and free-living work is (*by design*) the interaction of participants' environment with the basic physiological signals thought to dictate eating behaviours. This is particularly relevant as it has been suggested that in fact eating can

be seen as a behaviour more strongly influenced by environment than the individual (Cohen and Farley, 2008). During this intervention, analysis of feeding patterns indicates no differences between treatment groups in snacking or meal frequency (once the allotted morning breakfast is accounted for). As feeding occasions are seemingly unaffected by the exclusion of morning food intake it may be that those consuming breakfast subsequently ate "automatically" at perceived meal/snack times rather than in response to physiological cues to eat (Cohen and Farley, 2008). Previous work in free living individuals asked to record reasons for the initiation of feeding occasions showed that whilst hunger was reported on 21% of occasions, the statements "It was mealtime" and "Because of regular lifestyle" accounted for 46% of feeding occasions (Tuomisto et al., 1998), indicating a strong influence of habits upon feeding frequency.

The difference in physical activity energy expenditure between groups is in contrast to the best evidence previously available (Halsey et al., 2012). It is therefore important to note some of the key methodological differences between these studies. Crucially, Halsey and colleagues (2012) provided breakfast within the laboratory, where subsequently physical activity measurement commenced following completion of this meal at ~09:00 and was terminated at 17:00 each day. Not only does this method place restrictions upon the movements of participants but the limited recording period may reduce the sensitivity of the instruments to detect differences within different periods of the day. This is particularly relevant as the present results not only indicate lower overall (i.e. 24 h) physical activity energy expenditure but also highlight that the greatest impact was apparent during the morning.

Whilst there has been much attention given to the effect of physical activity upon energy intake, the opposite (i.e the effect of energy intake upon physical activity) has received very little attention. Previous studies that have measured energy expenditure with altered feeding frequency have failed to detect any differences in energy expenditure but have not been truly free-living due to the use of room calorimeters (Smeets and Westerterp-Plantenga, 2008; Verboeket-van de Venne et al., 1993; Dallosso et al., 1982; Taylor and Garrow, 2001) or incomplete/restrictive periods of measurement (Halsey et al., 2012).

Previous work in both rodent models and anorexia nervosa sufferers have reported elevated physical activity levels during severe energy deficit, termed starvation induced hyperactivity (Hebebrand et al., 2003). More recent results contradict these findings as long term calorie restricted diets between 10-30 % of energy needs were associated with reduced activity energy expenditure at follow up between 6-12 months of the interventions (Martin et al., 2011). While the impact of chronic energy restriction upon physical activity is receiving research attention, the impact of transient periods of energy restriction upon self-selected physical activity is yet to be fully investigated in humans. Whilst the current work can contribute to answering the fundamental question of whether acute periods of energy restriction are related to energy expenditure, future work should attempt to provide further information as to why this may be the case. For example, use of subjective scales of vitality given over the course of an intervention may further elucidate the impact of altered feeding patterns upon energy expenditure.

When comparing three versus seventeen feeding occasions a day, it has been reported that fasting total and HDL cholesterol concentrations were reduced in response to two weeks of the high feeding frequency pattern (Jenkins et al., 1989). At the other end of the feeding frequency spectrum, Stote et al. (2007) have reported increases in all measures of cholesterols when comparing individuals eating once (dinner) relative to three times a day. Additionally, two studies from the same group indicate that irregular feeding occasions and the omission of breakfast negatively affect fasting cholesterol concentrations (Farshchi et al., 2004b; Farshchi et al., 2005b). These changes have been speculated to be due to lower insulin levels resulting in less activation of the enzyme hydroxymethylglutaryl-coenzyme A, the rate limiting of cholesterol synthesis in the liver (Bhutani et al., 2010). These findings were not mirrored in our work. Extended daily fasting did not negatively impact fasting cholesterol levels, with no changes in response to the intervention apart from increased HDL cholesterol in both groups.

Insulin sensitivity measures calculated at baseline and following the 6 week intervention indicate that neither extended fasting nor daily breakfast consumption altered the response to an OGTT. For those participants in the breakfast group this is not unexpected, as for the majority of this group the intervention they were following

would not have been particularly dissimilar from their usual lifestyle. However, in those individuals fasting, the lack of impairment is a novel finding within the literature. Farshchi and colleagues (2005) have demonstrated a greater insulin response to a high carbohydrate test meal following two weeks of shifting the commencement of daily feeding back from 8am ~2-3 hours for two weeks. While the authors attribute stable fasting and postprandial glucose responses to the short term nature of the study the authors provide no potential mechanisms for the reported effects on insulin. However, they do suggest that the response may be specific to meal ingestion, so future investigations examining the response to meal ingestion following extended morning fasting interventions may reveal an impact upon postprandial insulin sensitivity.

In addition to the laboratory based measurements of insulin sensitivity, free-living measurements of glycaemic control were obtained during the intervention. While the majority of parameters obtained were not different between groups, it was observed that afternoon glucose variability was greater in the fasting than breakfast group despite no difference in eating occasions between the two groups from 12:00 onwards. This effect will potentially be partly attributable to the noted tendency for greater energy intake after 12:00 in the fasting group (341 kcal), and to the increase from a lower fasted concentration during the morning. However, it is interesting to note that this difference in afternoon/evening glucose variability was increased from week 1 to 6 of the intervention. This might suggest that chronic adherence to morning fasting may cause greater systemic glucose appearance or reduce disposal upon eating. It could be suggested that this may be a result of repeated exposure to greater glucose/insulin concentrations during the afternoon because of the absence of the associated reductions in these two metabolites to lunch following morning feeding (as demonstrated in Chapter 3). A further extension of this work to control any potential for confounding based upon energy intake/feeding patterns between groups would be to use continuous glucose monitors in laboratory settings with fixed composition/energy repeated feeding occasions to establish if either chronic breakfast consumption or morning fasting alters the glycaemic responses to repeated meals.

There are some limitations of the present work that must be considered when interpreting our findings. The nature of our study design for the free living measurements obtained during the study (i.e physical activity, energy intake) allowed

a simple between groups comparison. However, there was no assessment of these parameters of the two groups prior to commencement of the intervention. This was primarily due to practical considerations such as lab time/visits required and the potential for measurement fatigue in participants. This means it is not definitively possible to rule out that baseline differences between the groups account for the observed differences. However, whilst a "true" matched groups design was not feasible due to the rolling recruitment of participants; our two groups were very similar for all characteristics (i.e age, body composition, gender split, metabolic characteristics) measured at baseline. As such, there should be no reason to suspect such marked differences in free living outcomes as observed in the present work. Additionally, the most marked difference in this parameter was during the morning, coincident with the time of day that the conditions were implemented.

The choice to not dictate the foods consumed in the morning during the breakfast condition could be perceived as a potential drawback of the present work. However, this was deliberate as it was deemed that the least prescriptive intervention would give the best representation of "natural" compensatory responses. In addition, as the first study utilising certain measurement tools (i.e combined heart rate/accelerometry) to investigate a direct comparison of breakfast versus fasting, the largest practical dose was deemed the most appropriate to maximise the discrepancy between the two conditions. Within the context of the UK population where breakfast consumption is the norm (Reeves et al., 2013), the fasting condition can be seen as the "intervention", whereas the breakfast condition is closer to a "control". Whilst there is little scope for variation in what constitutes a morning fast, there are potentially limitless possibilities for a breakfast. Future work should focus on more prescriptive breakfasts to attempt to establish if specific permutations of macronutrient composition, morning feeding frequency or energy intake are necessary to confer health benefits.

The current finding of reduced body mass in those extending their fast is in contradiction to the majority of cross sectional associations and prospective studies. It is difficult to make meaningful comparisons with randomised trials in the extant literature, as most studies investigating altered feeding frequency have either been in the context of weight loss interventions (Schlundt et al., 1992; Keim et al., 1997) or

have deliberately attempted to maintain stable weight (Stote et al., 2007). In studies where weight change was neither prescribed nor prevented, no alterations in weight or body composition have been reported over 2 weeks (Farshchi et al., 2005b; Farshchi et al., 2005a). A recent 16 week study advising either breakfast skipping or consumption in overweight individuals did not result in differences in weight change between the two intervention groups (Dhurandhar et al., 2014a).

The observed mean weight change in those extending their morning fast over 6 weeks was modest (~400 g). It is well established that chronic energy deficit results in weight loss that slows over time due to changes in body composition, as the amount of body tissue that needs to be supported metabolically both at rest and during movement is reduced (Hall et al., 2011). It may be that the modest weight loss occurring over the current study period is not sufficient to induce compensatory mechanisms designed to protect (fat) mass, such as a reduction in leptin (Thong et al., 2000). Further studies over longer periods in lean individuals could provide information as to whether sustained weight loss is achieved through daily morning fasting, or if behavioural/hormonal modification over greater time results in differing weight change outcomes.

In summary, we conclude that in lean adults daily energy intake but also physical activity energy expenditure is lower in individuals extending their fast until 12:00 daily. Neither resting metabolic rate nor markers of cardiovascular health or metabolic control were substantially affected by either intervention. Contrary to epidemiological evidence, omission of breakfast did not lead to weight gain, but reduced physical activity associated with morning fasting may have negative longer term implications for health.

Chapter 5: Morning fasting for 6 weeks does not cause increased appetite, acute energy intake or negative metabolic consequences to feeding relative to daily breakfast consumption in lean adults

5.1 Introduction

Infrequent or insufficient breakfast consumption is associated with greater risk of overweight and obesity (Bazzano et al., 2005; Kant et al., 2008; Song et al., 2005). Cross-sectional evidence associating breakfast consumption and energy intake is equivocal, with some reports indicating no difference between breakfast consumers and skippers (Song et al., 2005; Wyatt et al., 2002) and others reporting increased energy intake in those that consume breakfast (Cho et al., 2003; Nicklas et al., 1998). Some evidence from randomised controlled trials is beginning to suggest that breakfast skipping leads to reduced energy intake (Reeves et al., 2014). Indeed, in Chapter 4 of this thesis we have reported lower free-living energy intake in those extending their morning fast, with evidence of stable energy intake over a 6 week intervention. However, the impact of interventions manipulating morning fasting upon acute energy intake, appetite and regulatory hormones has not been established. It remains to be seen whether chronic assignment to differing daily morning feeding regimens can affect short term appetite regulation (i.e whether prolonged exposure to daily morning fasting/feeding causes adaptation of responses measured acutely within a day).

A previous morning fasting intervention (Farshchi et al., 2005b) as well as the work described in Chapter 4 have examined health markers, and postprandial insulin sensitivity using a mixed macronutrient test drink (Farshchi et al., 2005b). In the study of Farshchi, Taylor and MacDonald (2005), the authors report reduced insulin sensitivity following a 2 week breakfast skipping intervention in lean women. In addition, the results from the intervention study reported in Chapter 4 have indicated some evidence for increased free-living glucose variability during the afternoon and evening in those that fasted daily from week 1 to 6 of the intervention. Whilst this was not associated with differences in glucose or insulin responses to an OGTT or changes in calculated indices of insulin sensitivity, it is important to establish if the morning

fasting regimen alters glycaemic control in response to repeated meals, as this is more similar to free-living feeding.

Neither of the aforementioned studies have examined if the appetite hormone responses to feeding are altered by adherence to either a daily breakfast or extended morning fasting regimen. Evidence from previous studies characterising appetite hormone responses to chronic (8 week) interventions based upon manipulating feeding frequency (but not the presence or absence of breakfast) (Cameron et al., 2010; Carlson et al., 2007) and macronutrient composition of diet (Ellis et al., 2012) have reported no adaptation of appetite hormone responses after the interventions.

The present study aims to investigate whether chronic (6-week) exposure to either a morning fasting or daily breakfast regimen can cause adaptations in acute appetite regulation and metabolic responses throughout the day, and energy intake at an *ad libitum* lunch in lean individuals. We hypothesise that appetite hormones will be unaffected by either morning feeding pattern but that postprandial insulin sensitivity will be reduced in those undertaking morning fasting for 6 weeks.

5.2 Participants and Methods

5.2.1 Participants

Thirty one healthy, lean men (n = 12) and women (n = 19) aged 22-56 y took part in this study. Participants were recruited via local advertisement from South West England and were initially assessed for eligibility based upon a body mass index of 18-25 $kg \cdot m^{-2}$ and then later classified as lean based upon DEXA-derived fat mass indices of $\leq$7.5 $kg \cdot m^{-2}$ (men) and $\leq$11 $kg \cdot m^{-2}$ (women)(Kelly et al., 2009). The study was part of a larger randomised controlled trial entitled the Bath Breakfast Project. In accordance with full eligibility criteria set out in Chapter 2, participants reported being weight stable (± 2% body mass within past 6 months) and adhering to a standard sleep-wake cycle (e.g no shift workers) and not anticipating any change in lifestyle during the study period. Participants were free of metabolic disorders, with pre-menopausal female participants either menstruating regularly, or following their chosen contraceptive method (i.e pill, implant) for >6 months. Within the study cohort there was a mix of regular breakfast consumers (classified as >50 kcal intake within 2 hours of waking on ≥4 days of the week) and non-consumers. Characteristics of participants are presented in Table 5.1.

Written informed consent was obtained from all participants, with the Ethical Approval for the study obtained from the NHS Bristol Research Ethics Committee. The study is registered with Current Controlled Trials [ISRCTN31521726].

Table 5.1: Participant characteristics

Characteristic		Breakfast Group	Fasting Group
n		15	16
Age (y)		36 (12)	35 (10)
Body Mass (kg)		66.6 (8.4)	66.0 (7.9)
Body Mass Index (kg/m^2)		21.6 (1.7)	22.7 (2.3)
Fat Mass Index (kg/m^2)*	**All**	5.1 (1.9)	5.7 (2.3)
	Female	6.1 (1.8)	6.7 (2.1)
	Male	3.6 (1.0)	4.1 (1.6)
Habitual Breakfast Consumers (*n*)		11	14
Female (*n*)		9	10

*Fat mass index calculated as DEXA derived total fat mass divided by height squared. Values represent mean with (SD)

5.2.2 Study Design

Each participant first undertook a laboratory based breakfast feeding trial in the Human Physiology Laboratories at the University of Bath. Following these visits participants then commenced one of two morning feeding interventions (breakfast consumption or morning fasting for a 6 week period-described in more detail below) and then returned to the laboratory to repeat their laboratory based breakfast consumption trial (Figure 5.1). Here, we report the effect of the participants' assigned intervention upon their energy intake, appetite and hormonal responses to the acute breakfast consumption trial.

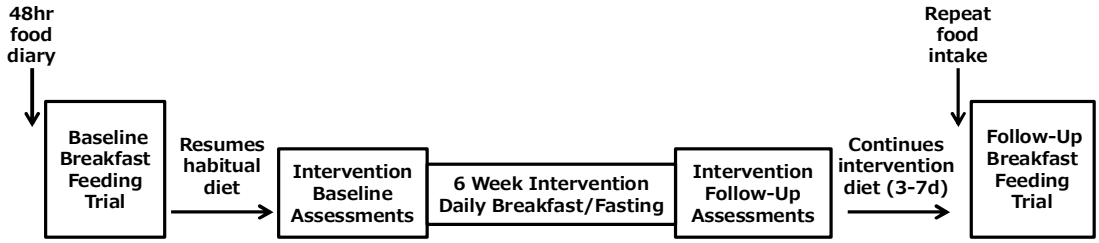

Figure 5.1: Schematic showing study design. In menstruating women the 6 week intervention commenced 2 weeks after the baseline assessments so that the intervention follow up visit was conducted in the luteal phase (3-10 days after onset of menses). In some cases the first breakfast feeding trial occurred after this baseline assessment but prior to the intervention beginning such that it was also within the luteal phase. The 48 h food diary completed prior to the first breakfast feeding trial and repeated prior to the follow up breakfast feeding trial conformed to the participants' assigned intervention.

5.2.3 Intervention

During the 6-week intervention, participants were randomised (1:1 allocation ratio) into either a group prescribed an energy intake of ≥700 kcal before 11:00 daily, with at least half consumed within 2 h of waking (Breakfast Group) or a group to extend their overnight fast by abstaining from ingestion of energy-providing nutrients (i.e. plain water only) until 12:00 each day (Fasting Group). Beyond the morning period all other lifestyle choices were allowed to vary naturally. The effect of this intervention upon components of energy balance and other health measures has been reported in Chapter 4. The randomisation of participants was stratified according to baseline breakfast habits to facilitate a more even distribution of habitual breakfast consumers and non-consumers in each group.

5.2.4 Standardisation of Participants Prior to Second Feeding Trial

Between the two laboratory visits, participants undertook their assigned dietary intervention for 6 weeks with pre- and post-intervention visits to assess body composition changes and glycaemic control (OGTT). Following this post-intervention visit, within 3-7 days, participants returned to the laboratory for their second breakfast feeding trial. During this intervening period participants continued to follow their assigned intervention dietary regime (i.e daily ≥700 kcal energy intake by 11:00 or 0 kcal until 12:00) such that any chronic adaptations to their feeding regime were not affected by a change of dietary practices before their follow up breakfast feeding trial (Figure 5.1).

5.2.5 Protocol for Laboratory Visits

The protocol during experimental visits is displayed in Figure 5.2. Participants reported to the laboratory at 08:00 ± 1 h, upon which adherence to standardisation measures as described in Chapter 2 were confirmed verbally. Participants then voided and had body mass measured in light clothing (Seca 873, Vogel and Halke). On their first visit to the laboratory prior to commencement of their intervention, following 20 minutes of quiet rest, resting metabolic rate was assessed in a supine position by repeated 5 minute expired gas samples over ~30 minutes, as according to best practice (Compher et al., 2006). Following their intervention on the second feeding trial, this procedure was repeated. A cannula was then inserted into an antecubital vein, with a baseline sample of 15 mL obtained and further samples acquired at regular intervals throughout the day. Participants were then provided with a breakfast, with blood samples taken at 15, 30 minutes and an hour post completion of the breakfast period. One hour after the breakfast period finished an expired gas sample was also obtained for assessment of dietary induced thermogenesis and substrate oxidation. Blood and gas samples were then obtained hourly until 3 h post breakfast, at which point an *ad libitum* lunch was provided. Upon completion of the lunch period (30 minutes), samples of blood and expired gas were obtained each hour for a further 3 hours. Participants also completed visual analogue scales relating to hunger and appetite throughout the day. During the day participants remained sedentary and completed quiet activities such as reading, watching television and typing. Full details of techniques and measurements employed are described in Chapter 2.

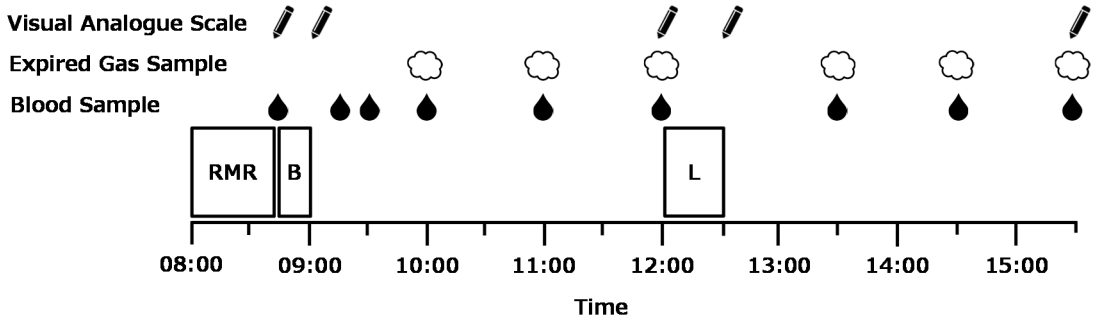

Figure 5.2: Schematic of experimental protocol. RMR=Resting Metabolic Rate, B=Breakfast consumption, L=*Ad libitum* lunch

5.2.6 Breakfast

The breakfast consisted of Corn Flakes (Kellogg's), 2% Fat Milk (Sainsbury's), toasted white bread (Braces), margarine (I can't believe it's not butter) and fresh orange juice (Sainsbury's) and was based upon the breakfast provided by Chryssanthopoulos and colleagues (2004). For provision of additional sugar, participants were given the choice of either white sugar added to cornflakes, or seedless raspberry jam (Sainsbury's) on their toast, or an iso-caloric combination of both. The overall percentages of energy from macronutrients in the breakfast were 70 % carbohydrate, 17 % fat and 13 % protein. The breakfast was provided in quantities that contained 0.06 g carbohydrate per kcal of each individual participants measured daily resting metabolic rate, resulting in energy intake of 472 ± 67 kcal and 460 ± 47 kcal in the breakfast and fasting groups, respectively. Quantities of the items provided for a typical breakfast are illustrated in Appendix 1. Participants were first provided with cereal, then at 5 minute intervals toast and finally orange juice, with all of the breakfast consumed within 15 minutes to standardise any effects of eating rate upon appetite hormones (Kokkinos et al., 2010).

5.2.7 *Ad Libitum* Lunch

Three hours post-breakfast participants were provided with an *ad libitum* lunch test meal consisting of 1 kg cooked (i.e wet weight) penne pasta (Sainsbury's) and tomato sauce (Ragu); prepared at a ratio of 1:1 uncooked mass. The overall percentages of energy from macronutrients for the lunch were 79 % carbohydrate, 14 % fat and 7 % protein. During their first trial participants were allowed *ad libitum* intake of plain water during lunch, this volume was subsequently replicated on their second visit. Participants were left alone during the lunch, with a recorded message

played prior to beginning consumption "We ask that you continue eating until you have satisfied your hunger. The lunch will remain in front of your for at least 30 minutes, at which point the post-lunch timer will be started, although you will be allowed to continue eating if you are still hungry." Pasta was provided in a large bowl containing 1 kg of cooked pasta which was replenished every 10 minutes during the lunch period to minimise any visual feedback relating to consumption volumes, and prevent any tendency to completely finish the portion provided. The weight of pasta consumed during the lunch was recorded and energy intake was calculated using manufacturer's nutritional information.

5.2.8 Statistical Analysis

For comparison of time series variables that were measured over the course of the day (e.g appetite hormones), 3-way mixed model ANOVA were employed to identify interactions independent of deviations from a normal distribution (Maxwell and Delaney, 1990) but with Greenhouse-Geisser corrections applied to intra-individual contrasts for $\varepsilon < 0.75$, and the Huynh-Feldt correction applied for less severe asphericity (Atkinson, 2002). ANOVA results are described using the following terms: a main effect of trial refers to a difference of the overall means from pre- to post-intervention (i.e across both intervention groups, irrespective of the time course within each day); a main effect of time refers to significant differences over the course of the testing day (i.e a change between time points within the day, irrespective of groups and trials); and a main effect of group indicates that the mean of both trials is different between the two experimental intervention groups (i.e the breakfast and fasting groups).

Where relevant, to provide a fuller appreciation of any potential changes in response to the interventions, time courses were also expressed as peak, nadir and fasting concentrations specific to each participant (e.g the average of the peak concentrations within each participant, irrespective of when this occurred), with these summary statistics subsequently analysed by 2-way ANOVA. For all statistical analysis, significance was accepted at $p \leq 0.05$. Data are presented in text as mean ± standard deviation, figures display mean with error bars representing the standard error of the mean (SEM). All statistical analyses were conducted using IBM SPSS statistics version 22 (IBM, New York).

5.3 Results

5.3.1 Mass and RMR

From their pre-intervention to post-intervention breakfast trial both groups body mass was stable within 0.35 kg. Resting metabolic rates prior to the intervention were similar between the fasting and breakfast groups (1402 ± 149 kcal·d^{-1} *vs* 1452 ± 201 kcal·d^{-1}) and were not differently affected by the intervention, such that the values after intervention for the fasting (1417 ± 129 kcal·d^{-1}) and breakfast (1421 ± 187 kcal·d^{-1}) groups were stable within 35 kcal·d^{-1} from pre- to post-intervention.

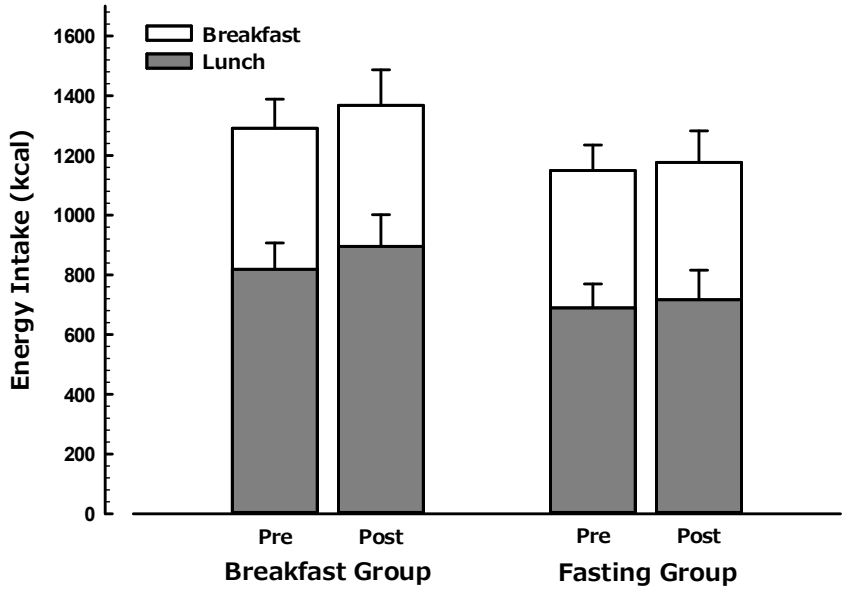

Figure 5.3: Energy intake during the feeding trial before and after 6-weeks of daily breakfast or fasting. Breakfast intake was prescribed, with the breakfast provided for both groups based on RMR similar (Breakfast Group, 472 ± 67 kcal *vs* Fasting Group, 460 ± 47 kcal; $p = 0.56$) and did not change from pre to post-intervention. The error bars on the grey portion of the stack represent the SEM of the energy intake at lunch, and the error bars on the white portion the SEM of the energy intake of the whole day.

5.3.2 Energy Intake

Energy intake at lunch was not different between groups or trials and there was no evidence of a group x trial interaction (all $p > 0.2$). Lunch intake was stable within 80 kcal in both groups. Overall intake including the prescribed breakfast was 1150 ± 341 kcal pre-intervention and 1177 ± 422 kcal post-intervention in those that fasted for 6 weeks. In those consuming breakfast for 6 weeks, intake was 1291 ± 377 kcal prior to the intervention and 1368 ± 461 kcal following the intervention.

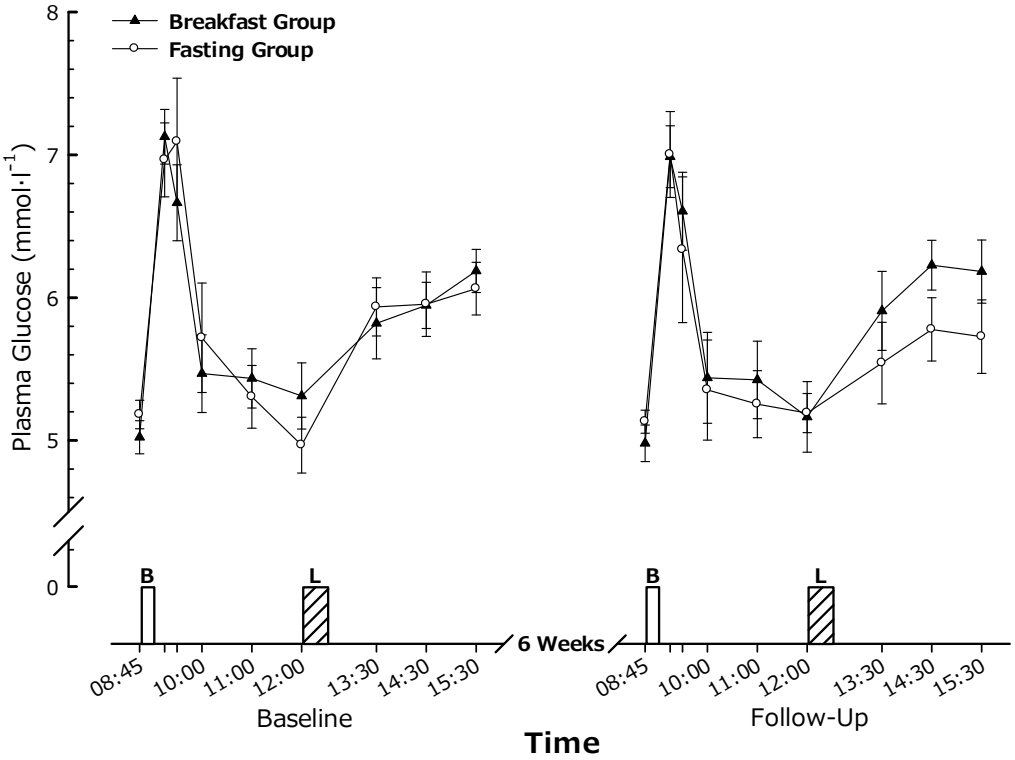

Figure 5.4: Glucose responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.3 Glucose

There was a main effect of time ($F = 22.2$, $p < 0.01$; Figure 5.4), but no main effects for group or trial and no interactions evident for blood glucose concentrations (all $p > 0.2$). Individual peak glucose concentrations were not different between groups or trials (Breakfast Group, 7.50 ± 0.73 mmol·l^{-1} *vs* 7.38 ± 0.85 mmol·l^{-1}; Fasting Group, 7.67 ± 1.27 mmol·l^{-1} *vs* 7.36 ± 1.30 mmol·l^{-1}) and there was no interaction effect (all $p > 0.2$). Similarly there were no main effects or interactions for individual nadir glucose (all $p > 0.3$), which remained stable in response to the intervention (Breakfast Group, 4.64 ± 0.59 mmol·l^{-1} *vs* 4.54 ± 0.76 mmol·l^{-1}; Fasting Group, 4.40 ± 0.59 mmol·l^{-1} *vs* 4.36 ± 0.62 mmol·l^{-1}). Fasting glucose was not different between trials or groups and there was no interaction effect (all $p > 0.2$).

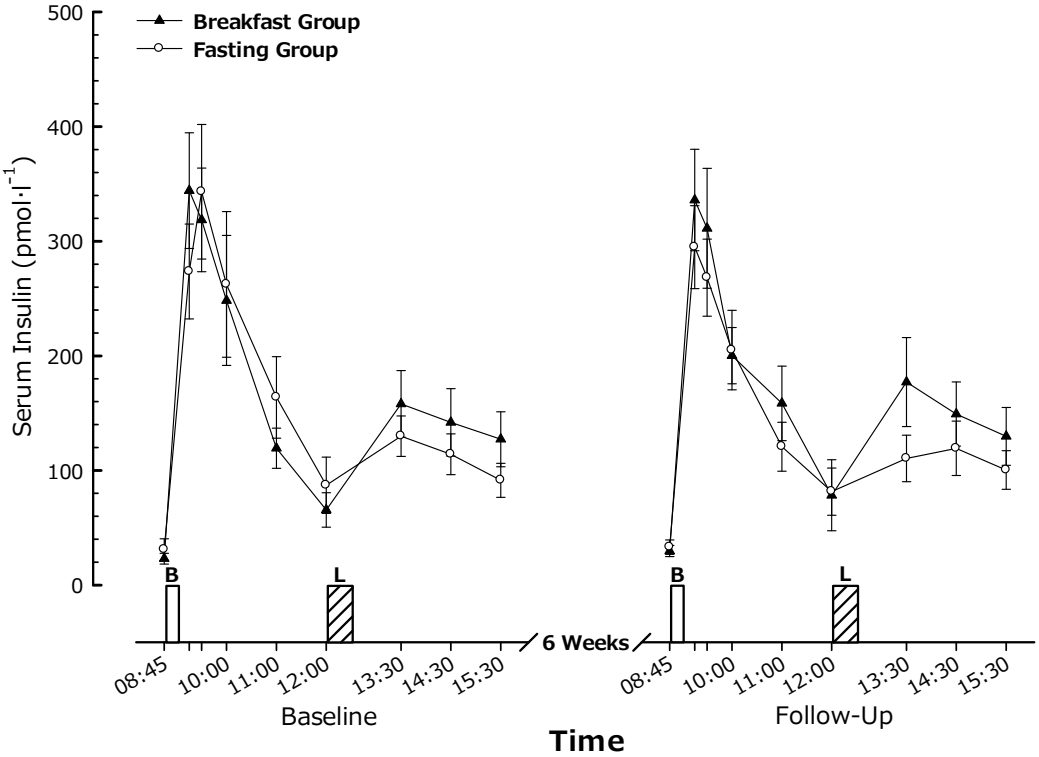

Figure 5.5: Insulin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.4 Insulin

Serum insulin concentrations displayed a main effect of time ($F = 41.0$, $p < 0.01$; Figure 5.5), but no effect of trial ($F = 0.18$, $p = 0.68$) or group ($F = 2.6$, $p = 0.12$). There were no interactions for the insulin response over the day (all $p > 0.1$). Individual peak insulin concentrations were not different between groups or trials (Breakfast Group, 401 ± 221 pmol·l^{-1} *vs* 374 ± 211 pmol·l^{-1}; Fasting Group, 336 ± 181 pmol·l^{-1} *vs* 352 ± 130 pmol·l^{-1}) and there was no interaction effect (all $p > 0.3$). Fasting insulin concentrations were not different between groups and there was no interaction effect (both $p > 0.6$) but there was a main effect of trial ($F = 5.05$, $p = 0.03$). Fasting insulin increased, across both groups, in response to the intervention (Breakfast Group, 23 ± 18 pmol·l^{-1} *vs* 30 ± 19 pmol·l^{-1}; Fasting Group, 31 ± 37 pmol·l^{-1} *vs* 33 ± 24 pmol·l^{-1}).

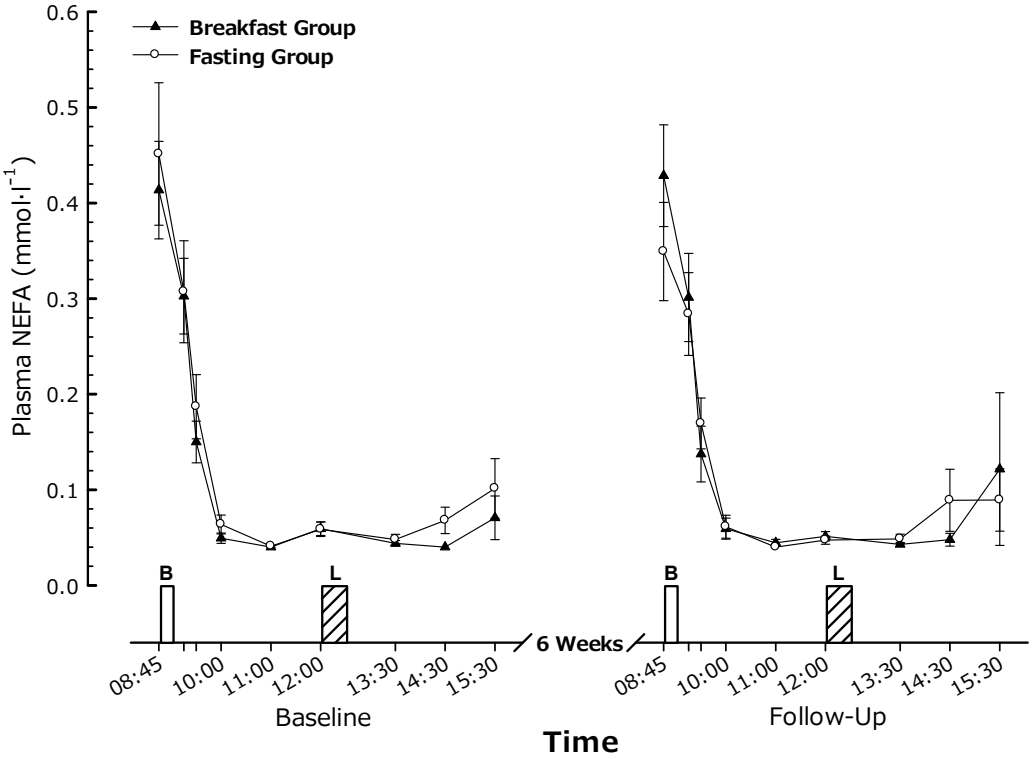

Figure 5.6: NEFA responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.5 NEFA

There was a main effect of time (F = 42.4, $p < 0.01$, Figure 5.6) for plasma NEFA concentrations throughout the day, with no effect of group, trial or any interactions (all $p \geq 0.1$). Fasting NEFA concentrations were not different between groups (F = 0.12, $p = 0.7$) but there was a main effect of trial (F = 4.24, $p = 0.05$) and a trial x group interaction (F = 4.33, $p = 0.05$). This interaction for fasting NEFA was primarily driven by a reduction in the fasting group (0.45 ± 0.29 mmol·l^{-1} *vs* 0.35 ± 0.20 mmol·l^{-1}; $p = 0.04$), with the breakfast group remaining stable (0.41 ± 0.19 mmol·l^{-1} *vs* 0.43 ± 0.21 mmol·l^{-1}; $p = 0.98$). Individual peak NEFA concentrations were similar between groups and trials (Breakfast Group, 0.43 ± 0.18 mmol·l^{-1} *vs* 0.47 ± 0.24 mmol·l^{-1}; Fasting Group, 0.48 ± 0.26 mmol·l^{-1} *vs* 0.38 ± 0.19 mmol·l^{-1}), and groups did not respond differently to the intervention period (all $p > 0.1$).

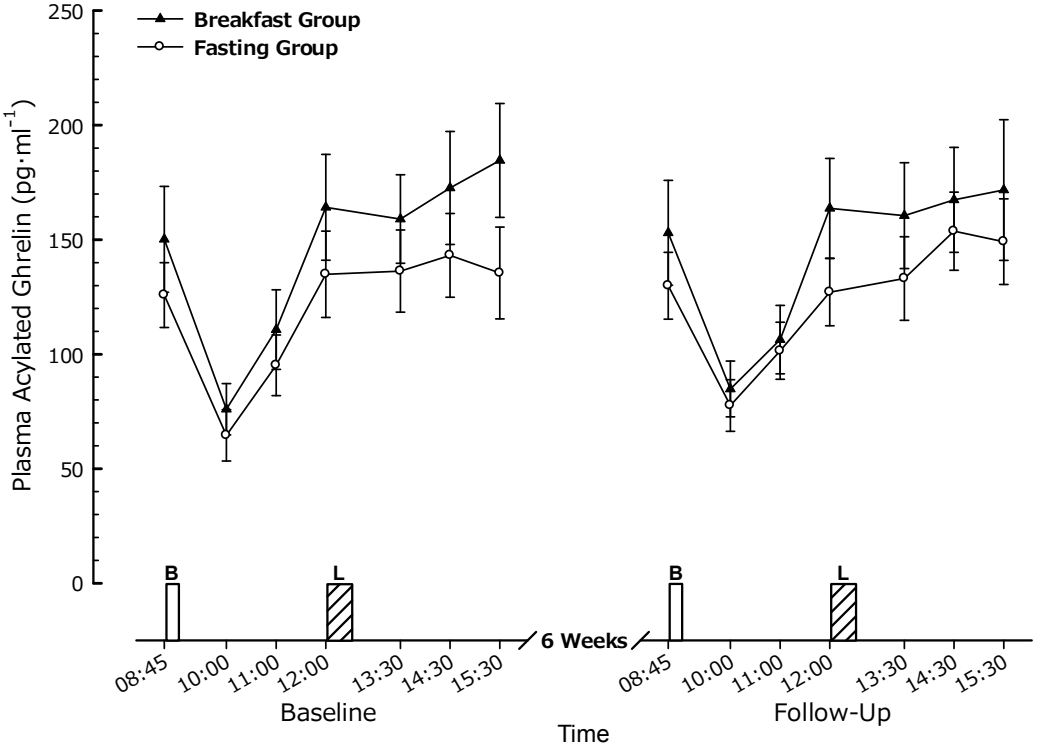

Figure 5.7: Acylated ghrelin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

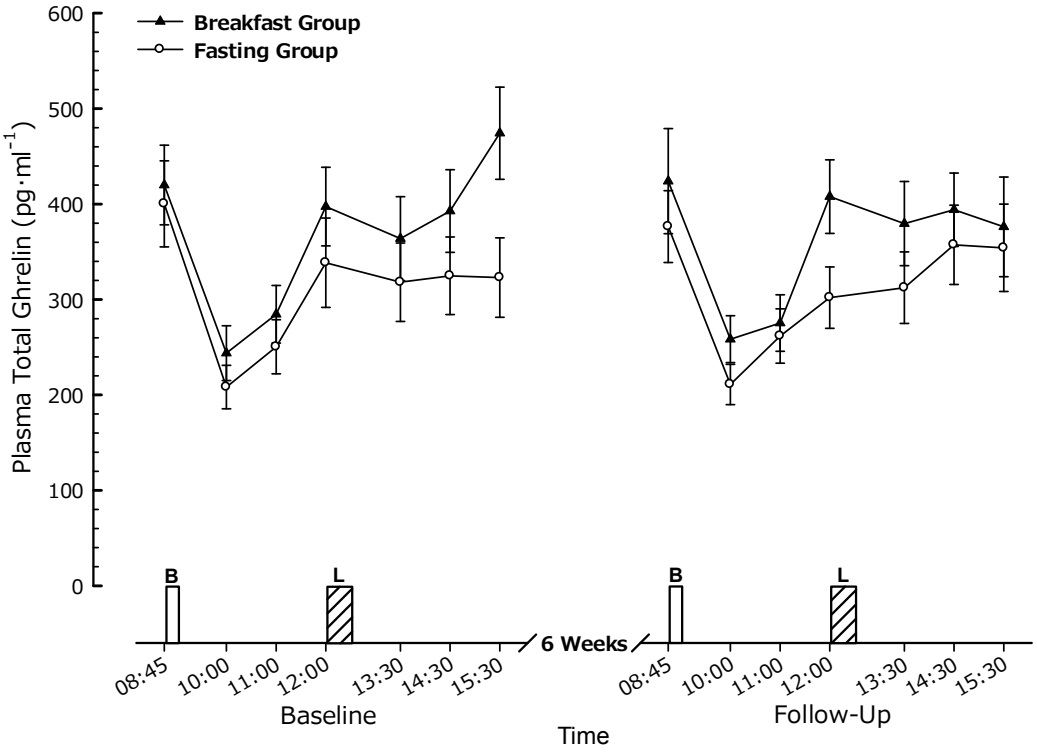

Figure 5.8: Total ghrelin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.6 Acylated and Total Ghrelin

Plasma acylated and total ghrelin concentrations are displayed in Figures 5.7 and 5.8. There was a significant effect of time ($F = 40.7$, $p < 0.01$) for acylated ghrelin concentrations over the day. There was no difference between groups or trials, and no interaction effects (all $p > 0.3$). Fasting acylated ghrelin was not significantly different between groups ($F = 0.6$, $p = 0.46$) but there was a tendency for a main effect of trial ($F = 3.53$, $p = 0.07$) as both groups had slightly increased fasting concentrations (Breakfast Group, 150 ± 86 pg·ml^{-1} *vs* 153 ± 89 pg·ml^{-1}; Fasting Group, 126 ± 55 pg·ml^{-1} *vs* 130 ± 57 pg·ml^{-1}) but without any interaction effect ($F = 0.37$, $p = 0.55$). Individual peak acylated ghrelin concentrations were not significantly different between groups or trials (Breakfast Group, 192 ± 96 pg·ml^{-1} *vs* 192 ± 106 pg·ml^{-1}; Fasting Group, 168 ± 77 pg·ml^{-1}*vs* 166 ± 74 pg·ml^{-1}) with no interaction effect (all $p > 0.4$). Individual nadir acylated ghrelin concentrations were not significantly different between groups and there was no interaction effect (both $p > 0.6$). However, there was a main effect of trial ($F = 4.7$, $p = 0.04$) for nadir acylated ghrelin, such that concentrations increased in response to both breakfast consumption (76 ± 43 pg·ml^{-1} *vs* 82 ± 45 pg·ml^{-1}) and fasting interventions (67 ± 45 pg·ml^{-1} *vs* 76 ± 44 pg·ml^{-1}).

There was a main effect of time ($F = 32.0$, $p < 0.01$) but no effect of group or any other interaction effects (all $p > 0.2$) for total ghrelin. Individual peaks of total ghrelin concentrations were not significantly different between groups or trials (Breakfast Group, 489 ± 206 pg·ml^{-1} *vs* 459 ± 211 pg·ml^{-1}; Fasting Group, 435 ± 168 pg·ml^{-1} *vs* 422 ± 177 pg·ml^{-1}) with no interaction effect (all $p > 0.2$). Similarly, there were no main effects or interactions evident (all $p > 0.3$) for individual nadirs of total ghrelin concentrations (Breakfast Group, 241 ± 110 pg·ml^{-1} *vs* 240 ± 108 pg·ml^{-1}; Fasting Group, 208 ± 87 pg·ml^{-1} *vs* 206 ± 73 pg·ml^{-1}). Fasting total ghrelin was unaffected by the intervention and was not different between groups (Breakfast Group, 420 ± 156 pg·ml^{-1} *vs* 424 ± 213 pg·ml^{-1}; Fasting Group, 400 ± 174 pg·ml^{-1} *vs* 376 ± 146 pg·ml^{-1}) with no interaction effect (all $p > 0.1$).

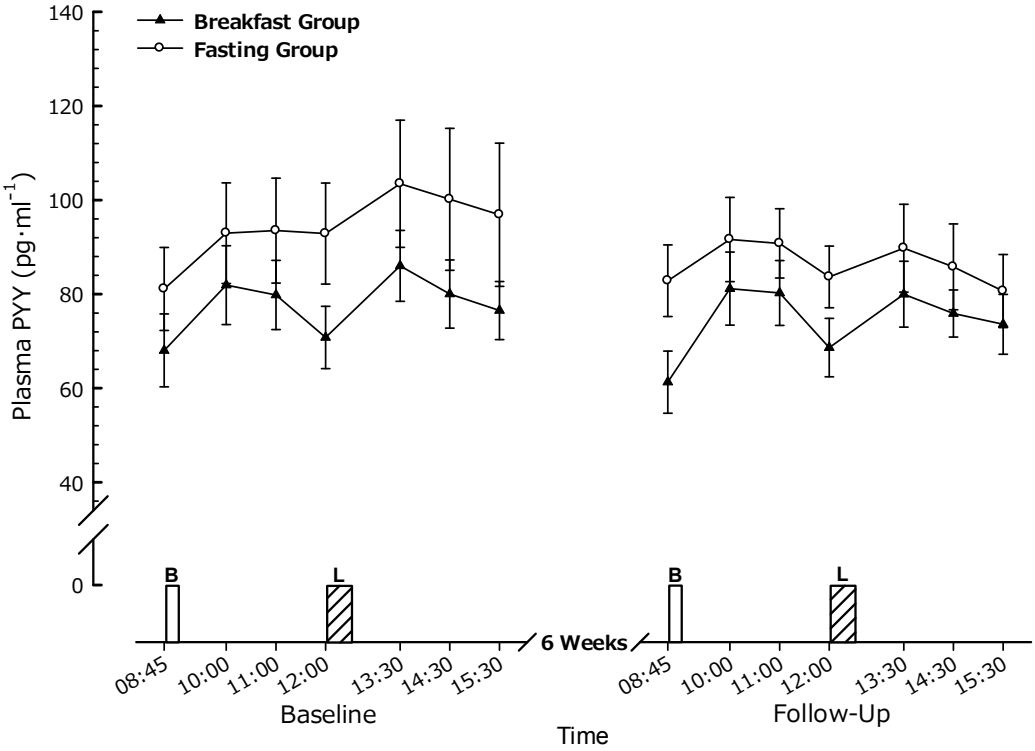

Figure 5.9: PYY responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.7 PYY

PYY concentrations varied over the course of the testing day ($F = 5.98$, $p < 0.01$; Figure 5.9). There was no effect of trial or group (both $p > 0.1$). A trial x time interaction was evident ($p = 0.04$) but no other interactions were apparent (all $p > 0.5$). There was no difference between groups or trials in fasting PYY (Breakfast Group, 68 ± 29 pg·ml^{-1} *vs* 61 ± 25 pg·ml^{-1}; Fasting Group, 81 ± 34 pg·ml^{-1} *vs* 83 ± 29 pg·ml^{-1}) or an interaction effect (all $p > 0.1$). Individual peak PYY was not different between groups ($F = 2.22$, $p = 0.15$) but there was a tendency for a main effect of trial ($F = 3.93$, $p = 0.06$), with peak PYY concentrations decreasing after the intervention in both groups (Breakfast Group, 97 ± 31 pg·ml^{-1} *vs* 92 ± 29 pg·ml^{-1}; Fasting Group, 124 ± 58 pg·ml^{-1} *vs* 107 ± 37 pg·ml^{-1}) but no interaction effect ($F = 1.22$, $p = 0.28$). Individual nadir PYY concentrations were not different between groups or trials (Breakfast Group, 58 ± 18 pg·ml^{-1} *vs* 53 ± 20 pg·ml^{-1}; Fasting Group, 71 ± 34 pg·ml^{-1} *vs* 68 ± 24 pg·ml^{-1}) with no interaction effect (all $p > 0.1$).

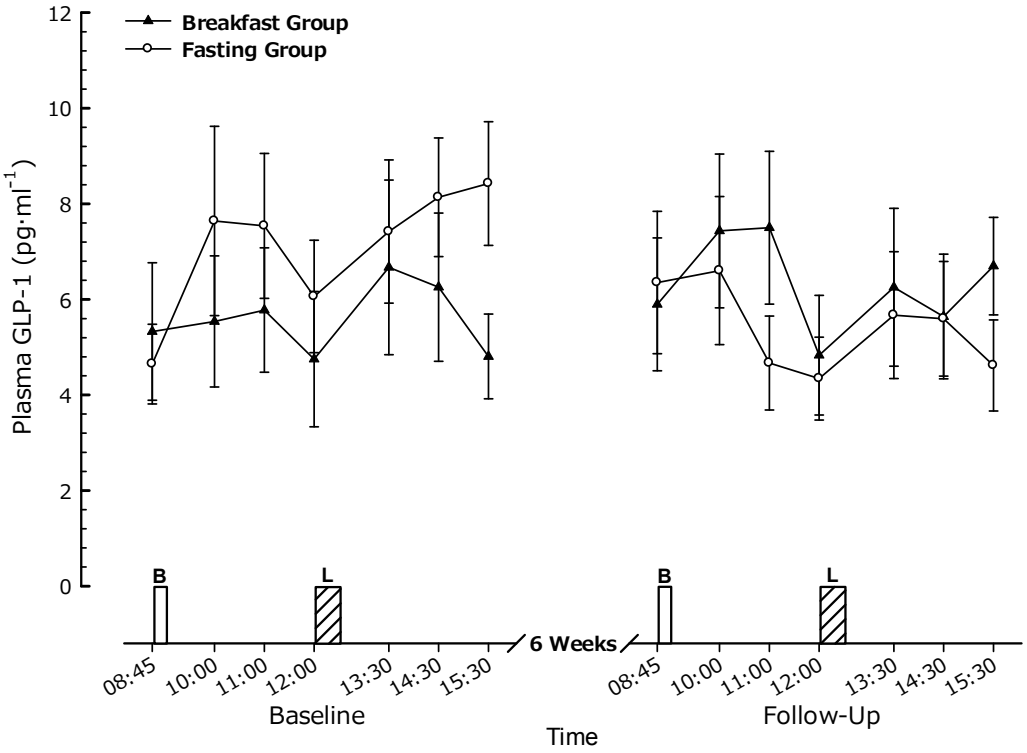

Figure 5.10: GLP-1 responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.8 GLP-1

There were no main effects of trial, group or time for GLP-1 (all $p \geq 0.1$; Figure 5.10). A trial x group interaction was apparent ($F = 4.17$, $p = 0.05$) but no other interaction effects were detected (all $p > 0.1$). Individual peak GLP-1 concentrations were not different between groups or trials (both $p > 0.3$) but there was an interaction effect ($F = 4.85$, $p = 0.04$) with an increase in the breakfast group (9.1 ± 6.4 pg·ml^{-1} *vs* 10.4 ± 6.2 pg·ml^{-1}) and a reduction in the fasting group (12.9 ± 8.0 pg·ml^{-1} *vs* 10.1 ± 6.5 pg·ml^{-1}).

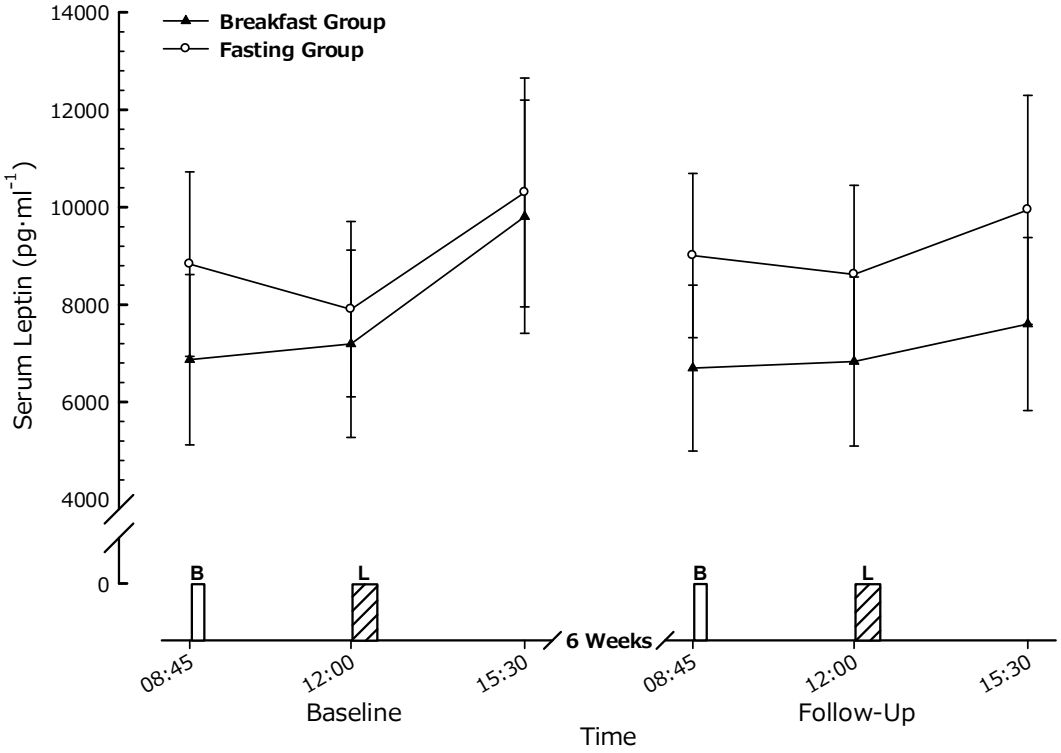

Figure 5.11: Leptin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad Libitum* lunch

5.3.9 Leptin

Leptin concentrations are displayed in Figure 5.11. A main effect of time was evident for leptin concentrations over the day ($F = 18.6$, $p < 0.01$) but no other main effects or interactions were detected (all $p > 0.2$).

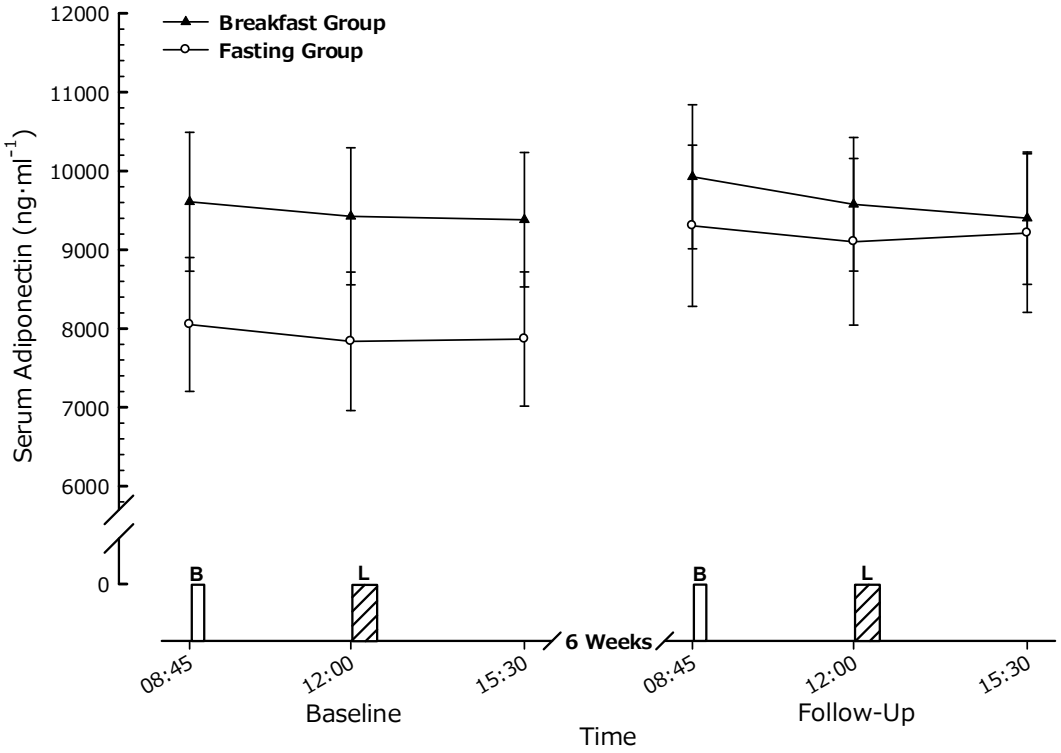

Figure 5.12: Adiponectin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.10 Adiponectin

Main effects of trial ($F = 4.65$, $p = 0.04$) and time ($F = 8.37$, $p < 0.01$) were apparent but groups were not significantly different for adiponectin concentrations ($F = 0.54$, $p = 0.47$, Figure 5.12). There were significant interactions of trial x group ($F = 4.17$, $p = 0.05$) and trial x time ($F = 3.37$, $p = 0.04$) but no other interactions (both $p > 0.6$). A main effect of trial for fasting adiponectin ($F = 6.4$, $p = 0.02$) was apparent but there were no effects of group or an interaction effect ($p > 0.1$).

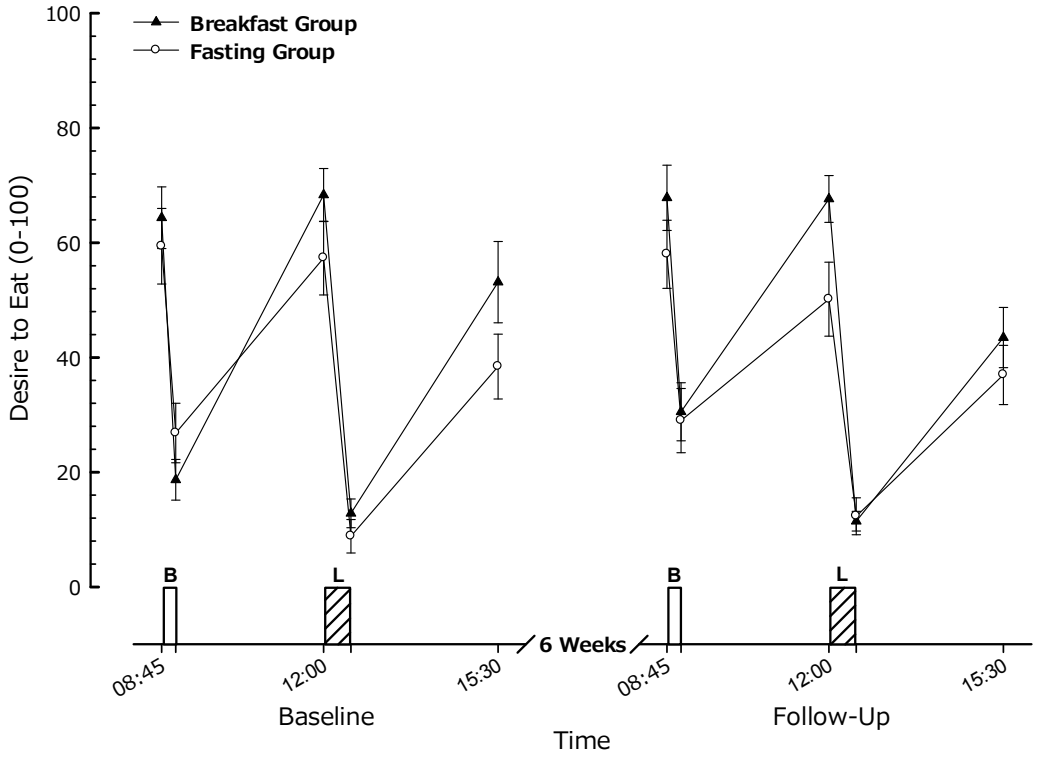

Figure 5.13: Desire to eat during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

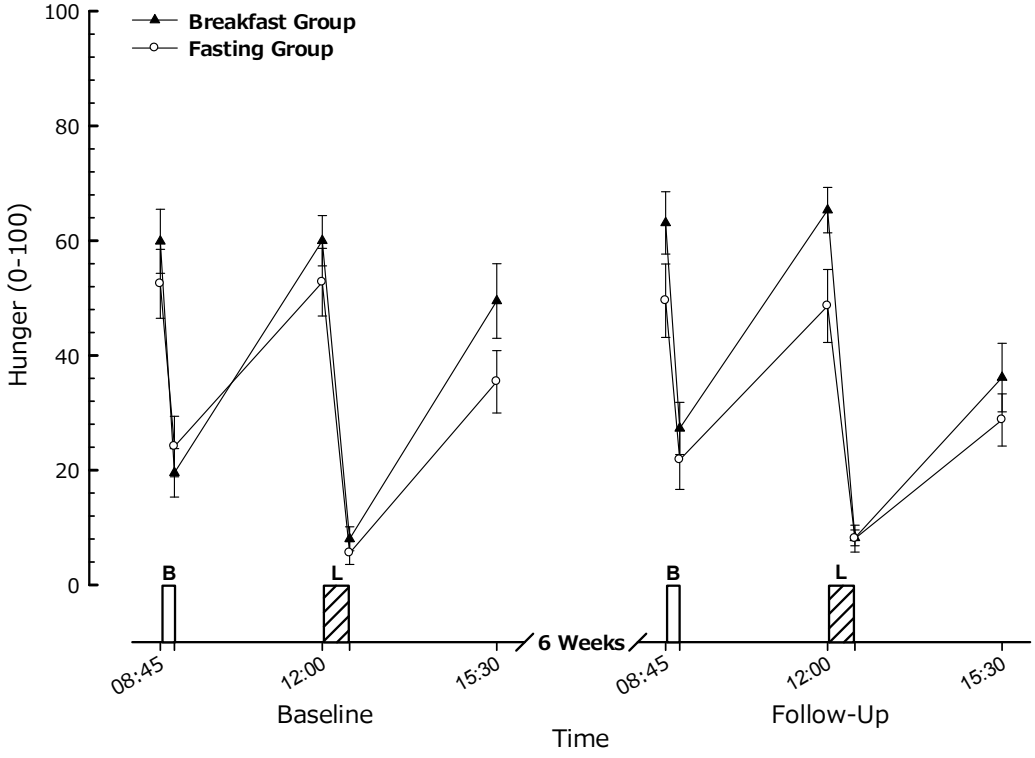

Figure 5.14: Hunger during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

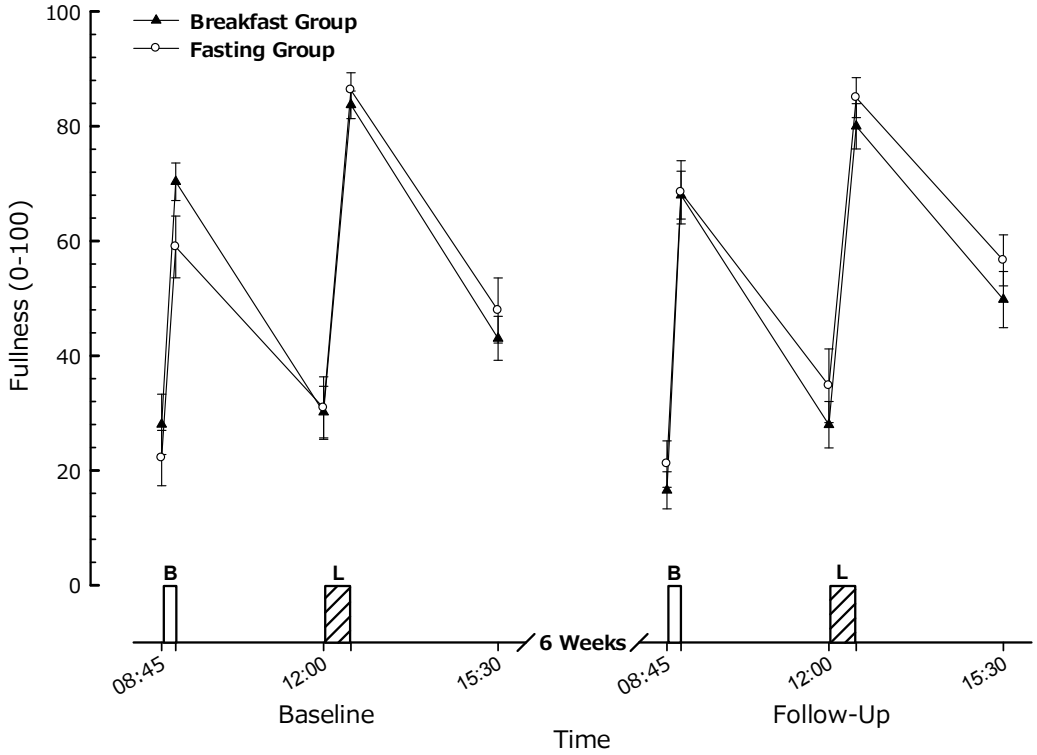

Figure 5.15: Fullness during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

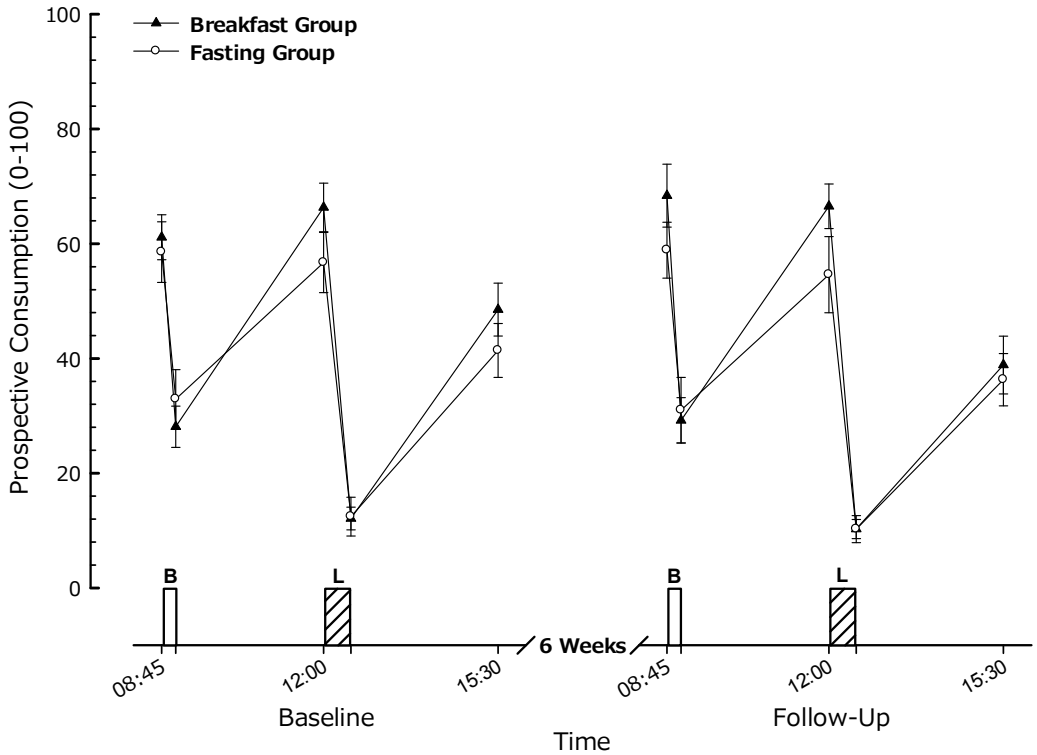

Figure 5.16: Prospective consumption during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

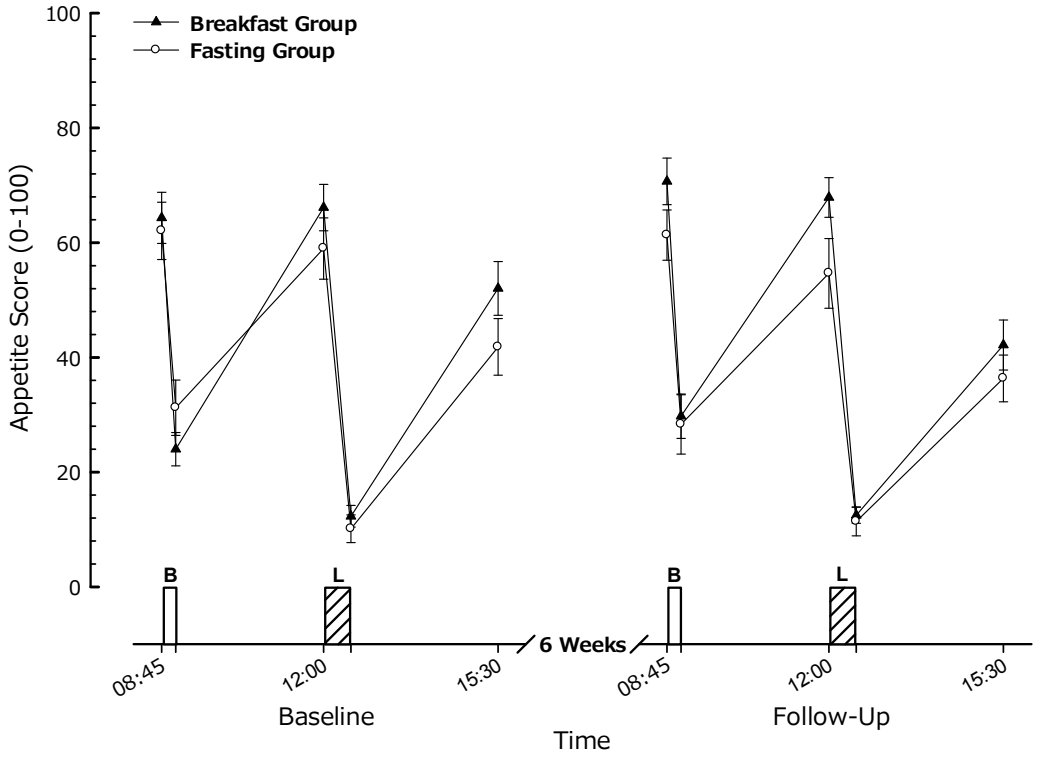

Figure 5.17: Appetite Score during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

5.3.11 Appetite Sensations

Appetite sensations are shown in Figures 5.13-17. There was a significant effect of time (F = 96.7, $p < 0.01$) but no main effect of trial or difference between groups (both $p \geq 0.3$) for desire to eat over the course of the day. There was a tendency for a time x group interaction ($p = 0.09$) but no other interaction effects (all $p > 0.1$). There were no main effects or interactions for fasting desire to eat (all $p > 0.2$). For hunger across the day, a main effect of time (F = 85.6, $p < 0.01$) but no difference between trials or groups (both $p \geq 0.2$) or any interactions were significant (all $p \geq 0.1$). There were no main effects or interactions for fasting hunger (all $p > 0.1$). Fullness varied over time (F = 138.9, $p < 0.01$), with no main effects of trial or group (both $p > 0.5$). A significant trial x group interaction (F = 4.50, $p = 0.04$) and a tendency for a trial x time (F = 2.57, $p = 0.06$) interaction were apparent, with no other interactions ($p > 0.2$). Fasting fullness was unaffected by trial, was not different between groups and there was no evidence of an interaction effect (all $p > 0.1$). There was a main effect of time (F = 109.8, $p < 0.01$) for prospective consumption but no other main effects or

interactions ($p > 0.2$). Similarly, no effects were apparent for fasting prospective consumption (all $p > 0.3$). The composite appetite score calculated from these sensations indicates that appetite varied over time ($F = 142.3$, $p < 0.01$) but there were no other main effects or interactions ($p > 0.1$). Fasting appetite scores were not different between groups or trials and no interaction effects were apparent (all $p > 0.2$).

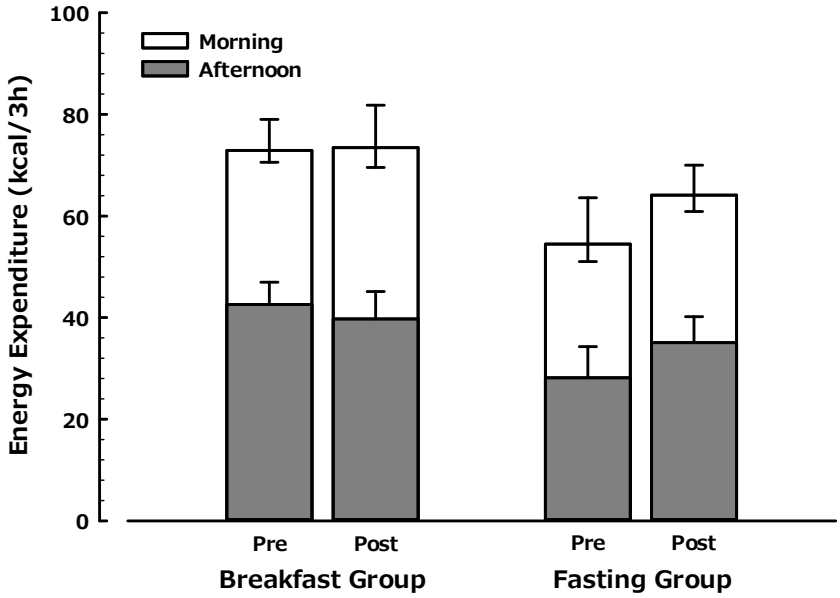

Figure 5.18: Diet induced thermogenesis during feeding trials before and after 6-weeks of daily breakfast or fasting. Error bars reflect SEM. Asymmetric error bars are plotted on the morning section of each stack. The negative portion of these error bars reflects the SEM of the morning period and the positive portion the SEM of the whole day.

5.3.12 Diet Induced Thermogenesis

There were no main effects of trial or group or an interaction of trial x group for morning DIT (all $p \geq 0.2$). Afternoon DIT was not different between trials or groups with no evidence of an interaction effect (all $p > 0.1$). When DIT was compared over the whole day there were no main effects or an interaction effect (all $p > 0.1$).

5.4 Discussion

The present study aimed to investigate acute energy intake, metabolic and appetite responses in a laboratory based protocol following a 6 week intervention of morning fasting or daily breakfast consumption in lean individuals. It was found that following a standardised breakfast, there was no adaptation of energy intake at an *ad libitum* lunch meal as a result of either daily fasting or breakfast consumption. As hypothesised, most appetite hormone responses were not differently regulated by either the fasting or feeding interventions. Contrary to our hypothesis, glycaemia and insulinaemia following feeding was not negatively affected by the fasting intervention. The present study indicates that a prolonged daily morning fasting intervention does not stimulate increased hunger, energy intake or negative metabolic consequences to acute feeding.

It has previously been reported that a 2 week period of breakfast eating in lean women reduced the insulin AUC to a mixed macronutrient drink whereas delaying the first feeding of the day until 11:00 h resulted in a significantly greater insulin profile following a test drink (Farshchi et al., 2005b). These findings have not been replicated in the current work. We found no evidence of either a significant reduction with breakfast consumption or detrimental increases in insulin response to feeding following 6 weeks of morning fasting. This is particularly interesting as the changes observed in the aforementioned work were induced in only 2 weeks of intervention, compared to the 6 weeks in the present study. These discrepancies in outcomes may centre upon differences in the cohorts studied (young women *vs* a mixed age and gender cohort in the current work). Additionally, there was a greater level of dietary control placed on participants, with a prescribed pattern of meals and snacks throughout the day that may contribute to the divergent findings.

The current work also examined the response to a second self-regulated feeding occasion. Although these meals were not identical pre- *vs* post-intervention, the magnitude of difference in both groups was an increase in EI of <80 kcal and as such allows a valid comparison of insulin response to relatively equivalent meals. Again, following this second meal there was no appreciable difference in insulin response during the afternoon. This confirms the postprandial insulin responses are not increased in individuals who followed a daily morning fasting intervention either

at the first meal of the day or having already eaten. It has previously been reported that acutely elevated NEFA concentrations can cause reduced insulin sensitivity due to impaired insulin signalling (Dresner et al., 1999), therefore it is interesting to note that NEFA concentrations throughout the testing day were also not increased following the fasting intervention.

To the best of the authors' knowledge, no study to date has investigated the effect of a sustained morning fasting intervention on hormonal factors regulating, and metabolic responses to, acute feeding (i.e if prolonged exposure to morning fasting causes adaptation of appetite responses examined within a day). With some authors having reported putative benefits for acute appetite regulation with breakfast consumption (Astbury et al., 2011; Leidy et al., 2013) our findings do not corroborate these previous reports as exposure to a morning fasting intervention did not lead to significantly increased intake at the *ad libitum* lunch. It could be contended that the nature of an *ad libitum* meal promotes overconsumption and therefore the test meal employed may not be sensitive to alterations in hunger. However, two pieces of evidence in combination lend credence to the lack of efficacy of the interventions in changing intake. Firstly, appetite ratings were not different prior to lunch following either intervention. Secondly, reported free-living energy intake in participants during the intervention reported in Chapter 4 were not different between weeks 1 and 6 of their assigned intervention; indicating no evidence of free-living dietary adaptation over time. This would seem to indicate that neither daily self-selected breakfast or more interestingly (as it was unaccustomed in more participants) morning fasting over a 6 week period induces any adaptations in acute energy intake or subjective appetite in lean individuals.

The absence of altered acute energy intake to either intervention is consistent with the stability of appetite hormone responses before and after the interventions. There have been few studies that have examined acute appetite hormone responses to feeding following a chronic dietary intervention. These investigations have examined the effect of interventions manipulating feeding frequency (Cameron et al., 2010; Carlson et al., 2007) and macronutrient composition of diet (Ellis et al., 2012) upon acute appetite hormone responses to feeding. In concordance with the current work, none of these prior investigations has reported alterations in acute appetite hormone

responses from pre- to post- chronic intervention. Although it has been reported that exercise programme (Martins et al., 2010) and diet induced (Cummings et al., 2002) weight loss interventions can modify postprandial appetite hormone concentrations, it appears that these responses are relatively resistant to change in the context of altered feeding patterns without substantial weight change.

One appetite hormone that has been suggested to be modifiable through altered feeding patterns is the orexigenic hormone ghrelin. Frecka and Mattes (2008) have suggested that ghrelin concentrations are entrained to habitual feeding patterns, with peaks coordinated with the expected time of feeding. Kim and colleagues (2012) have reported that when shifting breakfast intake from 07:00 to 09:00 for two weeks that the peak in ghrelin concentrations followed the change in eating time. Following this line of reasoning, it might be expected that ghrelin would peak later in the morning and/or be reduced at the start of the day following a fasting intervention but this was not the case in the current intervention. This may well be due to the duration of the overnight fast resulting in a "maximal" drive to eat independent of habitual patterns and potentially due to expectancy of feeding in the lab environment. A future line of enquiry would be to establish in time blinded individuals if the pattern of ghrelin release in the morning is altered following a morning fasting regimen.

Adiponectin concentrations significantly increased as a result of the fasting intervention, a finding that was not replicated in the breakfast group. It has previously been shown that adiponectin is inversely related to weight (Matsubara et al., 2002) and that weight loss via changes to diet and physical activity increase adiponectin concentrations (Esposito et al., 2003). However, when reviewing the evidence pertaining to caloric restriction interventions resulting in weight loss, Klempel and Varady (2011) have not reported a consistent increase in adiponectin. Studies of short duration involving moderate energy restriction for 4 days (Imbeault et al., 2004) or acute fasting for 48 hours (Gavrila et al., 2003) also do not increase adiponectin levels. In contrast with these findings are reports adiponectin increases in response to short term (5-7 days) overfeeding (Cahill et al., 2013a; Walhin et al., 2013; Brons et al., 2009), with some authors suggesting that this increase may be a counter regulatory response to counter insulin resistance in the short term (Brons et al., 2009; Cahill et al., 2013a). It may be that in the fasting group an increase in adiponectin reflects a

similar response to attempt to preserve insulin sensitivity. It should also be acknowledged that the fasting group had lower initial adiponectin concentrations and therefore the increase in this group could also represent some regression to the mean.

This study has found that a 6 week period of daily morning fasting does not cause adaptation of acute appetite regulation or increase acute energy intake. Appetite hormone responses to morning and lunch feeding were mainly unchanged by either a morning fasting or breakfast consumption regimen, indicating relative stability of responses in the absence of weight change as observed in this context. In conclusion, this work provides no evidence for a detrimental impact of daily morning fasting upon acute appetite regulation and metabolic responses to feeding in lean adults.

Chapter 6: Morning fasting does not stimulate increased energy intake at a lunchtime meal, but causes differing metabolic and hormonal responses relative to a typical breakfast in obese adults

6.1 Introduction

In Chapter 3 we characterised the energy intake, metabolic and appetite responses to acute breakfast consumption and morning fasting followed by an *ad libitum* feeding occasion in lean individuals. Whilst there is a relative lack of literature comparing these conditions in lean individuals, to the best of the authors knowledge no studies have compared the appetite hormone and metabolic responses to extended morning fasting *versus* breakfast consumption in obese individuals.

It cannot be assumed that the responses of lean individuals will be the same as obese individuals. The clearest indication that this should not be the case is that excess adiposity in obese individuals is not simply a physiological status but the result of a process of energy imbalance that has not been reversed over time. In contrast, lean individuals have demonstrated that they are able to manage long term energy balance effectively.

Previous studies contrasting obese individuals with lean counterparts have highlighted several relevant differences that might be expected to contribute to divergent findings in the two groups. It has been reported that obese individuals have delayed satiation to feeding, with greater energy intake before reaching maximum satiation (Delgado-Aros et al., 2004). This discrepancy in satiation may partly be influenced by differences in appetite hormones.

It has been established that several appetite hormones are either present in different concentrations in obese individuals or respond differently to feeding compared with lean individuals. Batterham and colleagues (2003) have reported that PYY concentrations were lower postprandially in obese than normal weight individuals despite greater intake during an *ad libitum* buffet meal. This attenuation of PYY release has been implicated in the reduced satiety induced by meals in obese individuals (le Roux et al., 2006). Ghrelin concentrations are lower in obese than lean

individuals (Tschop et al., 2001b; Shiiya et al., 2002). Additionally, the suppression of ghrelin in response to feeding has been reported to be both reduced (le Roux et al., 2005) and not present at all in obese individuals (English et al., 2002), with some evidence to suggest that nadir ghrelin concentrations after feeding are delayed in obese men (Carroll et al., 2007).

When reviewing relationships between insulin, glucose and appetite regulation in test meal studies by meta-analysis, Flint and colleagues (2007) have reported inverse associations between insulin and both hunger and energy intake in lean but not overweight individuals. In addition, they also report a significant correlation between appetite sensations and energy intake at *ad libitum* meals in normal weight but not obese individuals.

With these pertinent differences between obese and lean individuals in mind, the aim of the present study is to examine the appetite, metabolic and hormonal responses to acute morning fasting and breakfast consumption in obese individuals. Due to previous reports of reduced satiety hormone concentrations and lesser suppression of ghrelin following feeding, it is hypothesised that breakfast consumption will not reduce intake at a subsequent feeding occasion in obese individuals.

6.2 Participants and Methods

6.2.1 Participants

Twenty four healthy, obese men (n = 8) and women (n = 16) aged 25-58 y took part in this study. Participants were recruited via local advertisement from South West England and were initially assessed for eligibility based upon a body mass index of ≥ 30 $kg \cdot m^{-2}$ and then later classified as obese based upon DEXA-derived fat mass indices of ≥9 $kg \cdot m^{-2}$ (men) and ≥13 $kg \cdot m^{-2}$ (women) (Kelly et al., 2009). The study was part of a wider randomised controlled trial (the Bath Breakfast Project). In accordance with full eligibility criteria outlined in Chapter 2, participants reported being weight stable (± 2% body mass within past 6 months) and adhered to a standard sleep-wake cycle (e.g no shift workers) and did not anticipate any change in lifestyle during the study period. Participants were free of metabolic disorders, with pre-menopausal female participants either menstruating regularly, or following their chosen contraceptive method (i.e pill, implant) for >6 months. Within the study cohort there was a mix of regular breakfast consumers (classified as >50 kcal intake within 2 hours of waking on ≥4 days of the week) and non-consumers. Characteristics of participants are presented in Table 6.1.

Written informed consent was obtained from all participants, with the Ethical Approval for the study obtained from the NHS Bristol Research Ethics Committee. The study is registered with Current Controlled Trials [ISRCTN31521726].

Table 6.1: Participant characteristics

Characteristic		
n		**24**
Age (y)		**44 (10)**
Body Mass (kg)		**96.7 (19.0)**
Body Mass Index (kg/m^2)		**33.5 (4.7)**
Fat Mass Index (kg/m^2)*	**All**	**13.4 (3.9)**
	Female	**15.1 (3.7)**
	Male	**9.8 (1.0)**
Habitual Breakfast Consumers (*n*)		**14**
Female (*n*)		**16**

*Fat mass index calculated as DEXA derived total fat mass divided by height squared. Values represent mean with (SD)

6.2.2 Study Methodology

The study design, experimental protocols and approach to statistical analysis for this investigation were identical to those outlined in Chapter 3 of this thesis. The only difference in this study was the quantity of breakfast provided. The breakfast was again provided in quantities that contained 0.06 g carbohydrate per kcal of each individual participant's measured daily resting metabolic rate and due to the greater body mass (and therefore correspondingly greater RMR) of the obese participants in this study, this resulted in an energy intake of 521 ± 94 kcal.

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6.3 Results

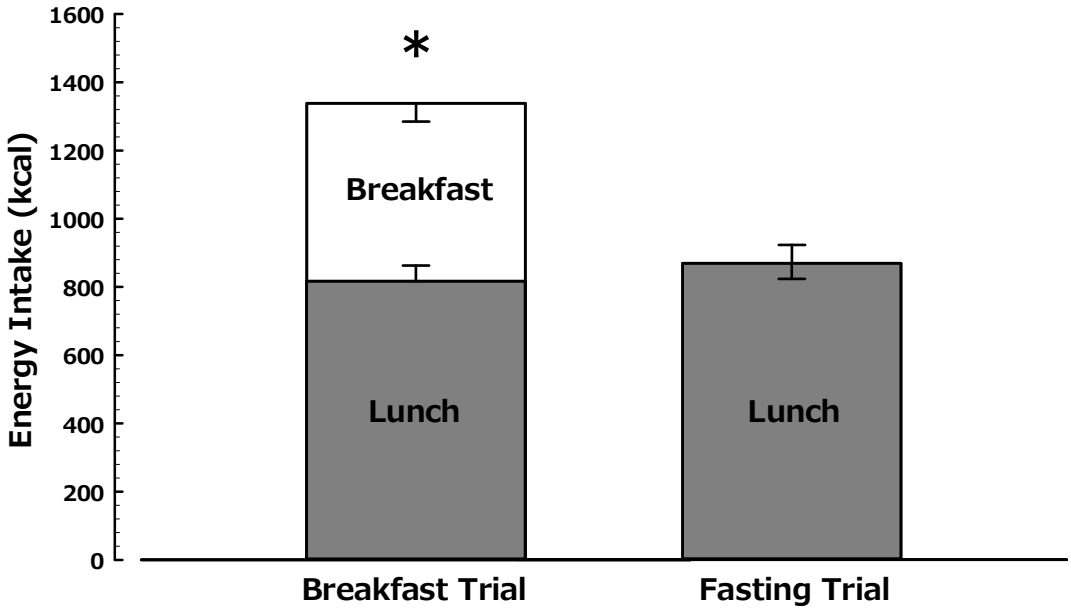

Figure 6.2: Energy intake during trials. In the fasting trial an asymmetric error bar is plotted. The positive portion of the error bar reflects the comparison between total intake during the fasting trial (i.e lunch only) and total intake on the breakfast trial, with the negative portion reflecting the comparison between lunches. * $p < 0.01$ for difference between total energy intake of breakfast versus fasting trial. *Equivalent figure for lean individuals on page 98*

6.3.1 Energy Intake

The energy content of the breakfast provided to participants was 521 ± 94 kcal (i.e variance proportionate to inter-individual differences in RMR). Energy intake at the *ad libitum* lunch was 817 ± 325 kcal in the breakfast trial and was not significantly different in the fasting trial (869 ± 354 kcal; $p = 0.1$). The additional intake at lunch during the fasting trial accounted for 10% of the intake provided with breakfast, resulting in a greater absolute intake over the testing day of 469 kcal ($p < 0.01$) during the breakfast trial.

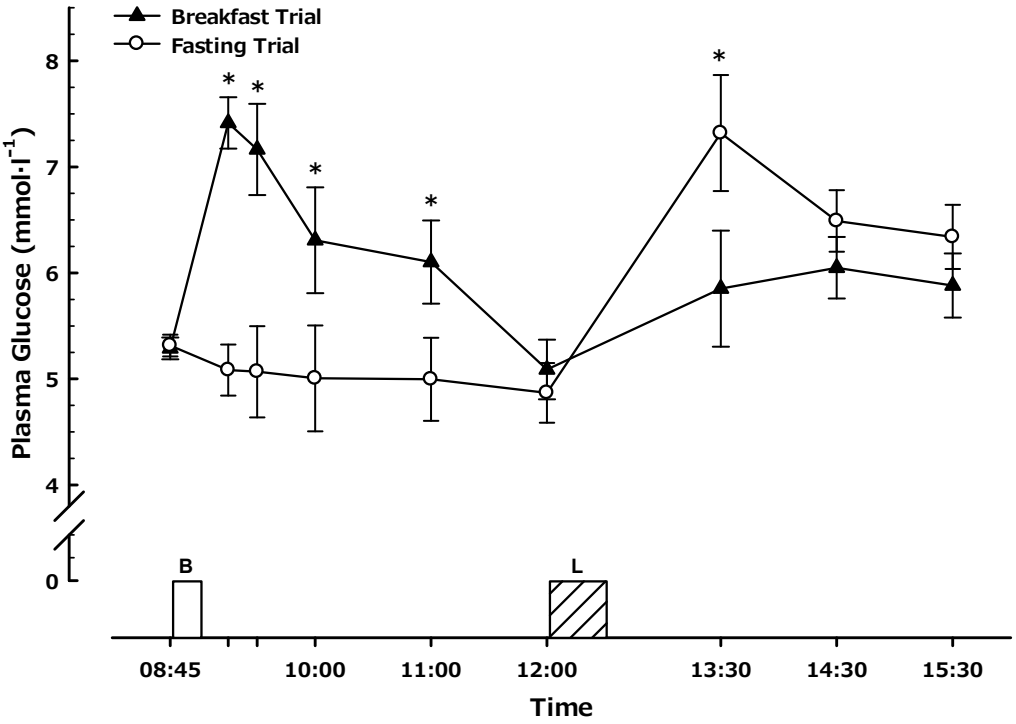

Figure 6.3: Glucose responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 99*

6.3.2 Glucose

There were main effects of trial and time, and an interaction of trial x time for blood glucose (all $p < 0.01$). Blood glucose was greater following breakfast consumption until 2 hours post breakfast (all $p < 0.01$; Figure 6.3) but was not different 3 hours after consumption ($p = 0.26$). Following lunch consumption, blood glucose concentrations were greater in the fasting trial 1 hour after lunch ($p < 0.01$) but not throughout the rest of the afternoon (both $p > 0.1$).

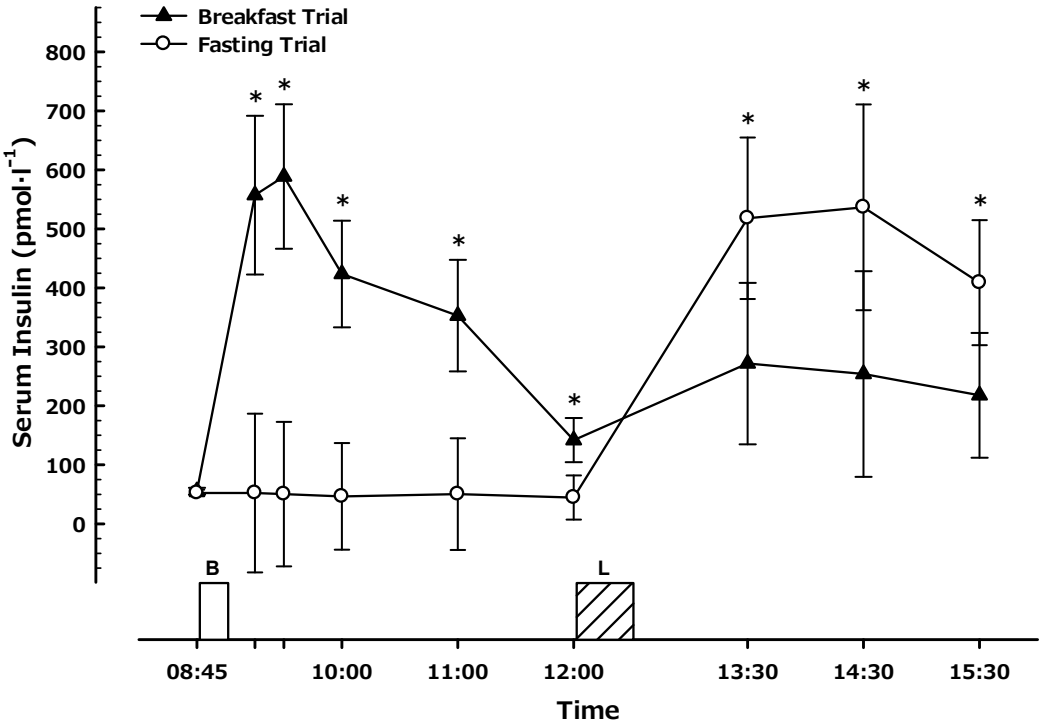

Figure 6.4: Insulin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p \leq 0.05$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 100*

6.3.3 Insulin

Similar to glucose concentrations, there were main effects of time, trial and a trial x time interaction for insulin (all $p < 0.02$). Insulin concentrations were greater throughout the morning following breakfast consumption (all $p < 0.02$, Figure 6.4). After the *ad libitum* lunch, insulin concentrations were greater in the fasting trial (all $p = 0.05$).

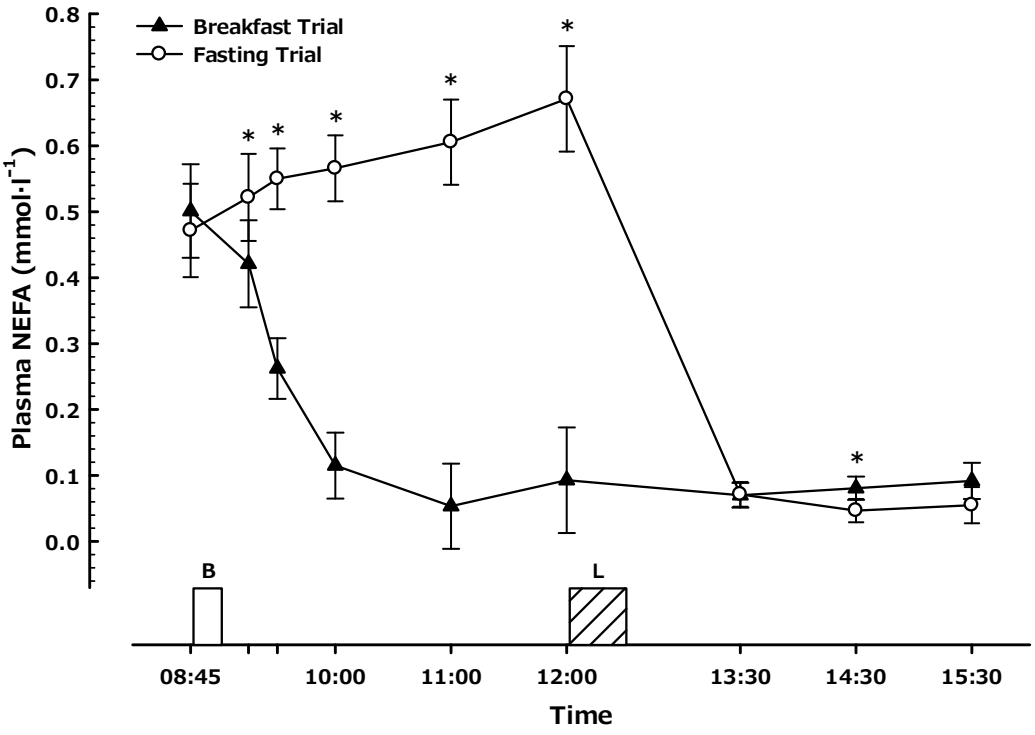

Figure 6.5: NEFA responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p \leq 0.05$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 101*

6.3.4 NEFA

Main effects of time and trial were evident for NEFA as well as a time x trial interaction (all $p < 0.01$). NEFA concentrations were reduced in the breakfast trial compared with the fasting trial from 15 minutes after breakfast ($p = 0.05$) and throughout the rest of the morning (all $p < 0.01$, Figure 6.5). There were no differences (both $p > 0.1$) between NEFA concentrations in the two trials at 1 and 3 hours after lunch consumption. There was a statistically significant but quantitatively small difference 2 hours after lunch consumption (Breakfast; 0.08 ± 0.06 mmol·l^{-1} *vs* Fasting; 0.05 ± 0.02 mmol·l^{-1}; $p = 0.04$).

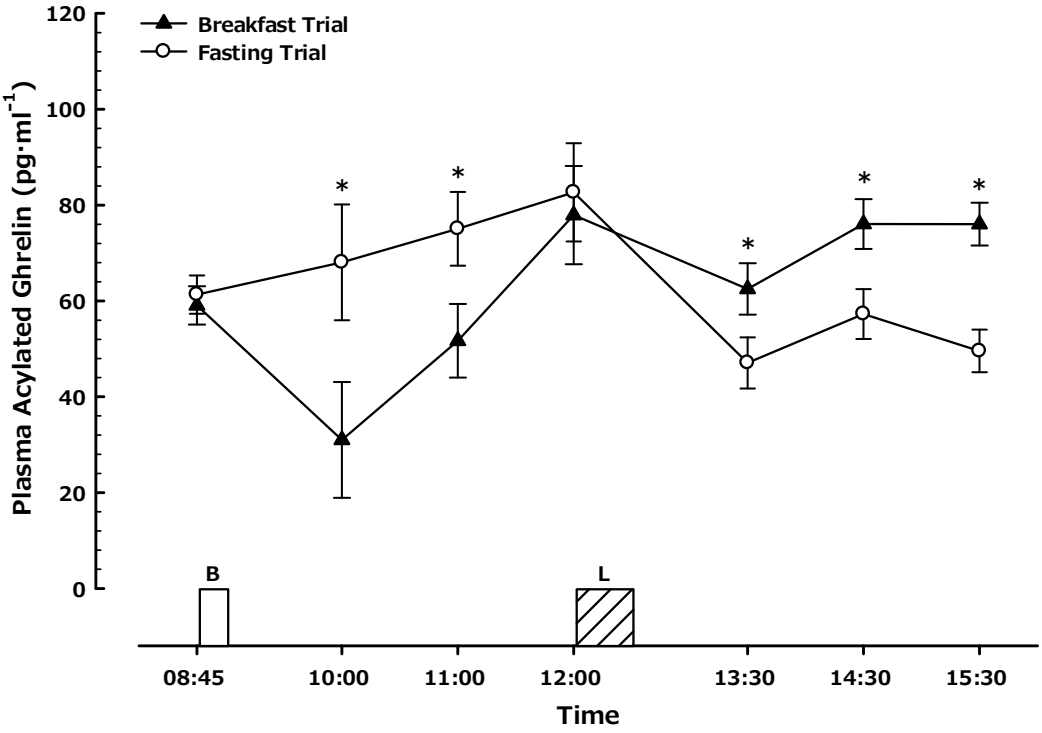

Figure 6.6: Acylated ghrelin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 102*

6.3.5 Acylated Ghrelin

A main effect of time and a trial x time interaction (both $p < 0.01$) were apparent for acylated ghrelin concentrations. Acylated ghrelin concentrations were lower following breakfast consumption until 2 hours post-breakfast (both $p < 0.01$; Figure 6.6) with no difference between the trials immediately prior to the *ad libitum* lunch ($p = 0.47$). Following lunch consumption, acylated ghrelin was suppressed below baseline concentrations throughout the afternoon in the fasting trial but remained above baseline in the breakfast trial (all $p < 0.01$).

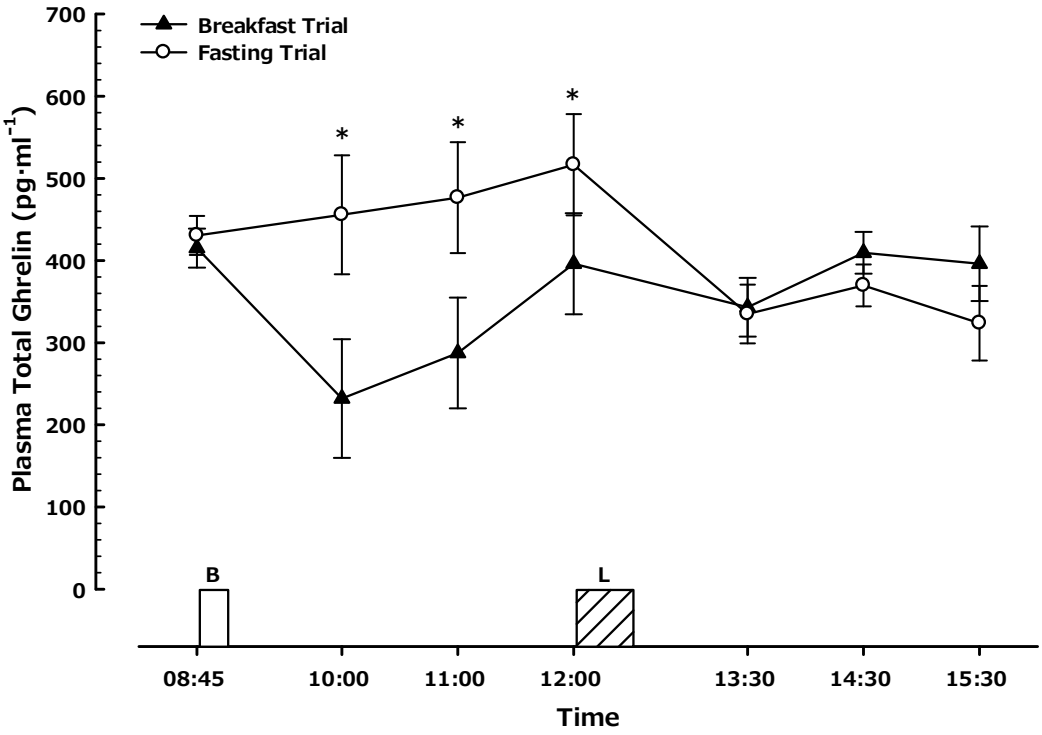

Figure 6.7: Total ghrelin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.04$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 102*

6.3.6 Total Ghrelin

There were main effects of trial, time and and a trial x time interaction (all $p < 0.01$) for total ghrelin concentrations. Total ghrelin concentrations were significantly lower than the fasting trial following breakfast consumption throughout the morning (all $p < 0.04$, Figure 6.7). Following lunch consumption there was no difference between total ghrelin concentrations in the two trials.

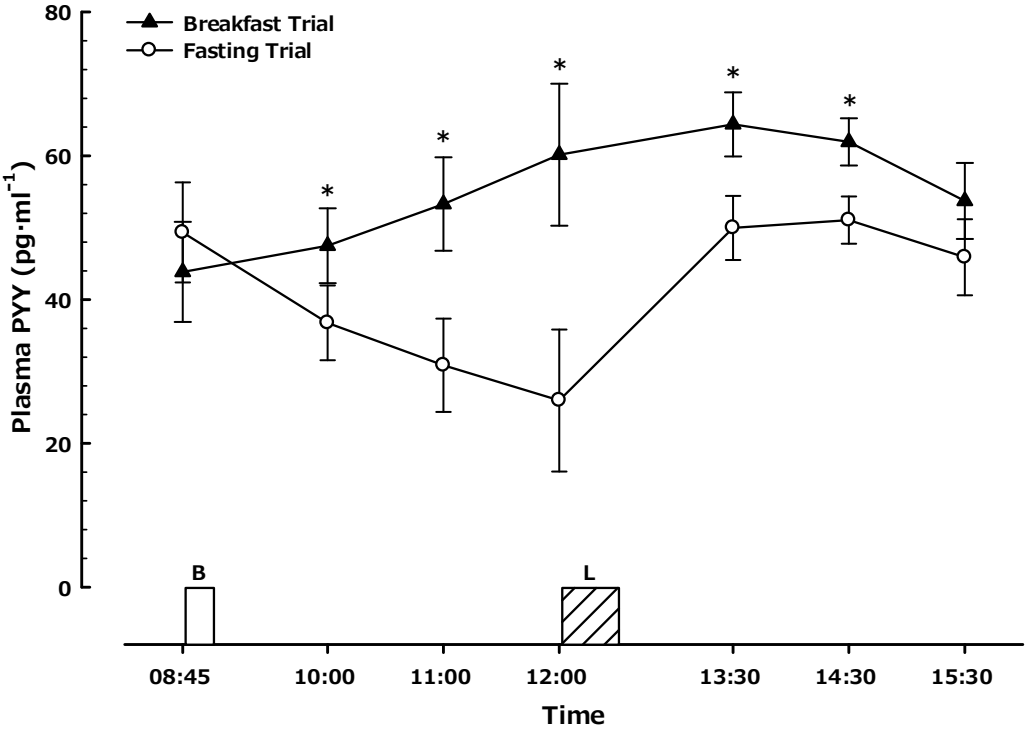

Figure 6.8: PYY responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.02$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 104*

6.3.7 PYY

PYY concentrations differed over time, between trials and there was a trial x time interaction evident (all $p < 0.01$; Figure 6.8). PYY concentrations were not different at baseline ($p = 0.25$) but from 1 hour after breakfast until 2 hours after lunch, PYY concentrations were greater in the breakfast trial (all $p < 0.02$). Three hours after lunch consumption, the difference between trials was reduced such that there was only a strong tendency for greater concentrations in the breakfast trial ($p = 0.06$).

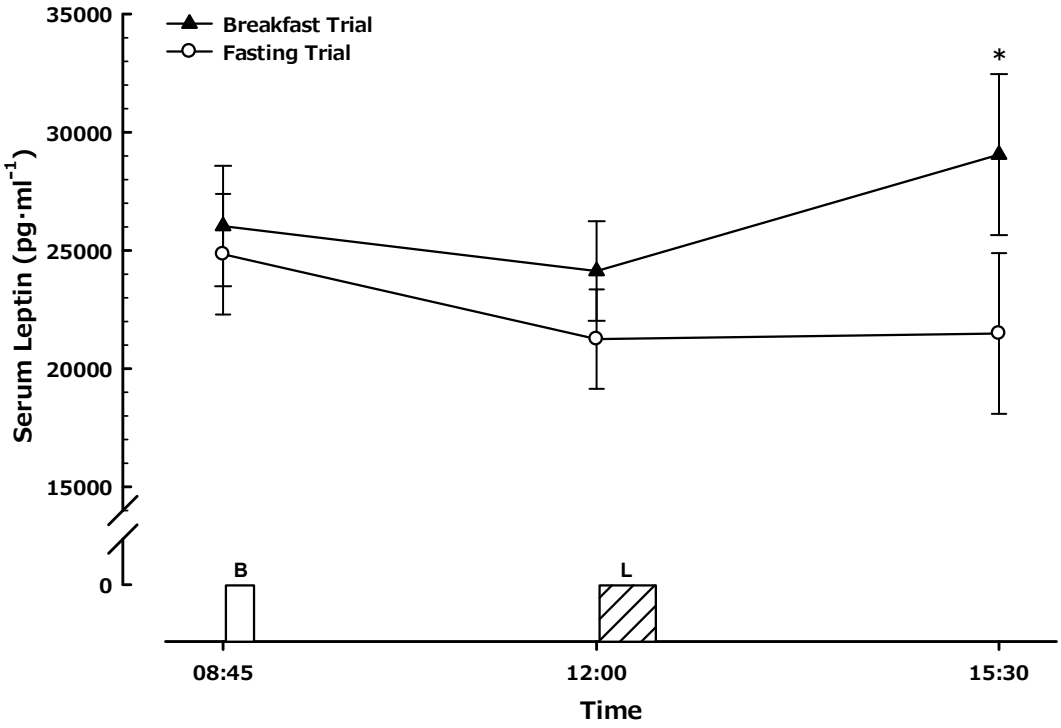

Figure 6.9: Leptin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial. *Equivalent figure for lean individuals on page 106*

6.3.8 Leptin

For leptin concentrations there were main effects of trial, time and a significant trial x time interaction (all $p < 0.04$; Figure 6.9). Leptin concentrations were not different at baseline ($p = 0.5$) and decreased pre-lunch in both groups, but with a tendency for lower concentrations following fasting (Breakfast Trial, 24133 pg·ml^{-1} vs Fasting Trial, 21252 pg·ml^{-1}, $p = 0.06$). Three hours after lunch consumption, leptin concentrations were significantly greater in the breakfast consumption trial (29059 ± 22968 pg·ml^{-1}) than the fasting trial (21489 ± 15346 pg·ml^{-1}; $p < 0.01$).

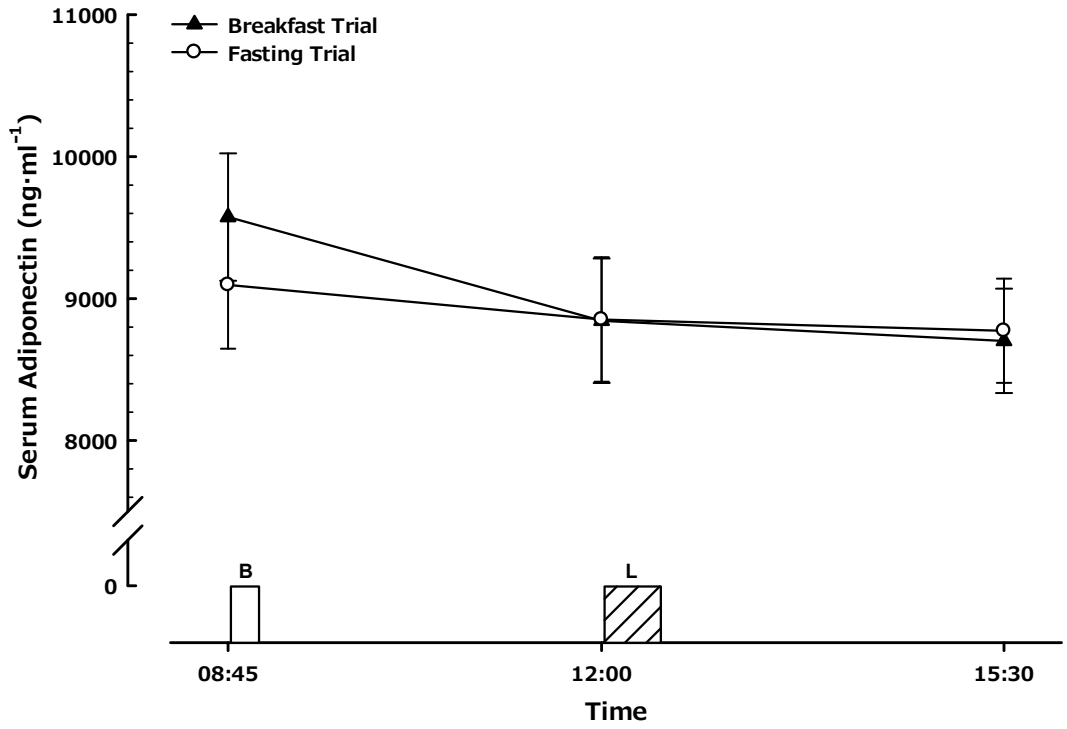

Figure 6.10: Adiponectin responses during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. *Equivalent figure for lean individuals on page 107*

6.3.9 Adiponectin

Adiponectin concentrations over the day displayed a main effect of time ($F = 6.47$, $p < 0.01$). No other main effects or interactions were apparent (all $p > 0.2$, Figure 6.10).

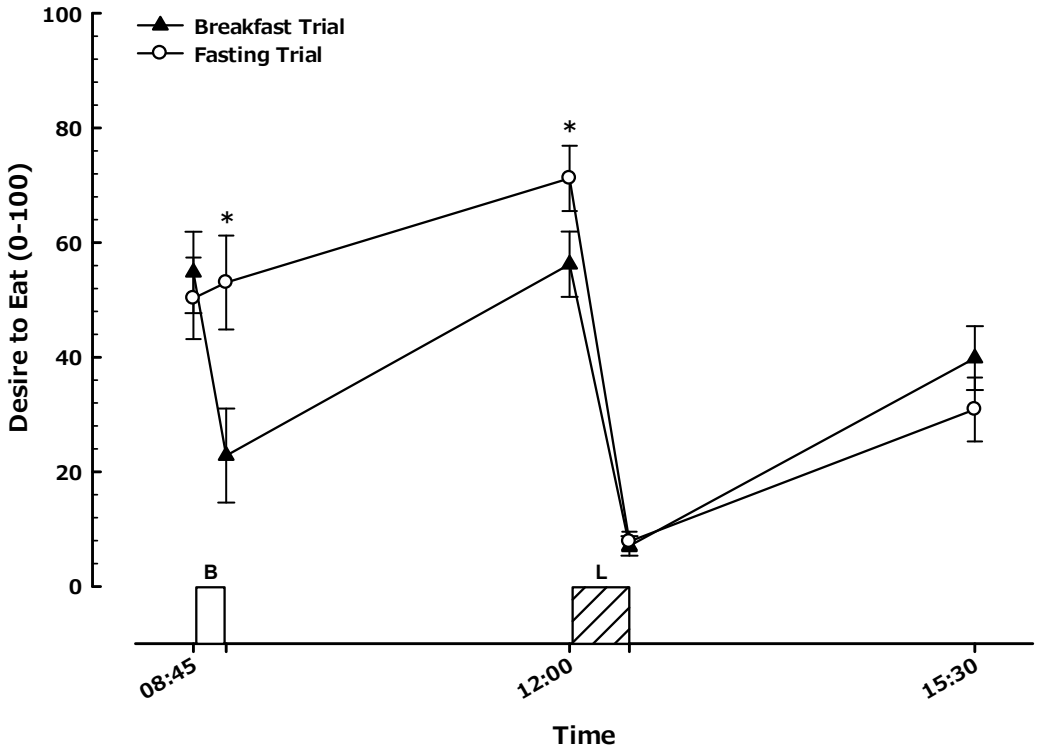

Figure 6.11: Desire to eat during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

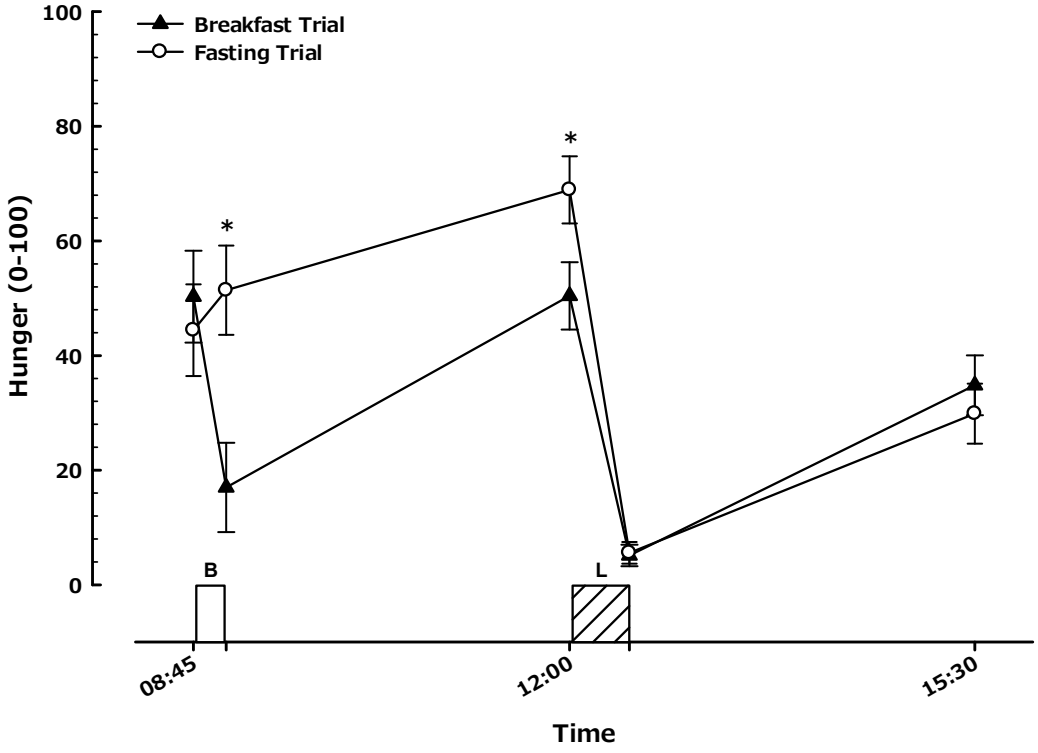

Figure 6.12: Hunger during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

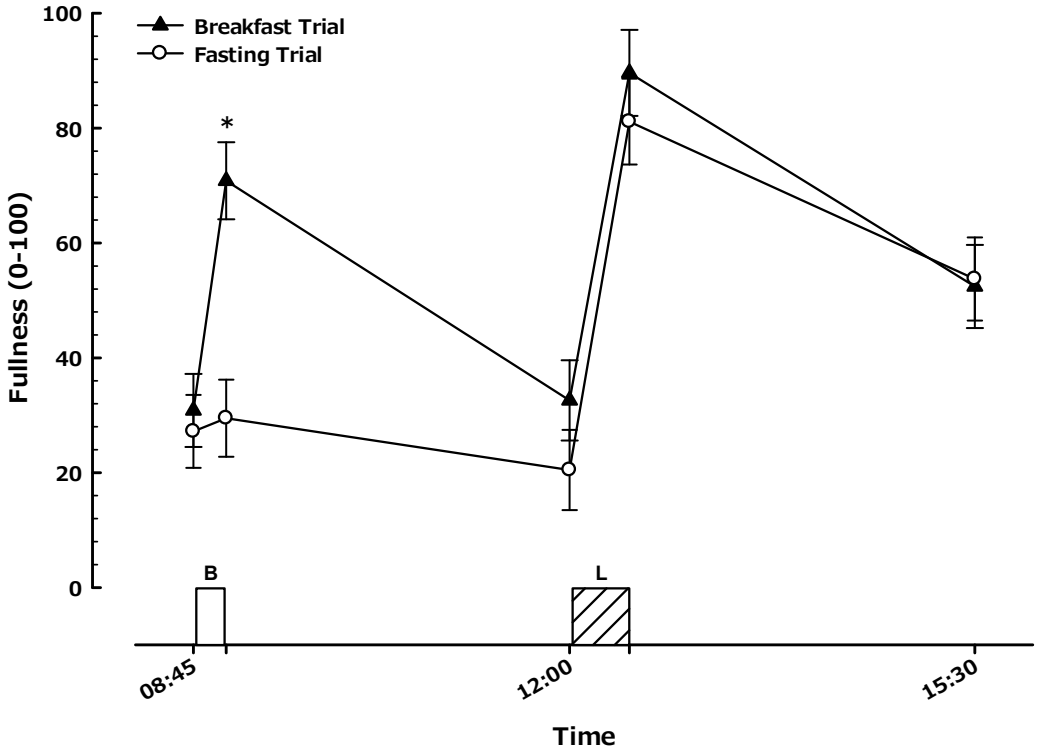

Figure 6.13: Fullness during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

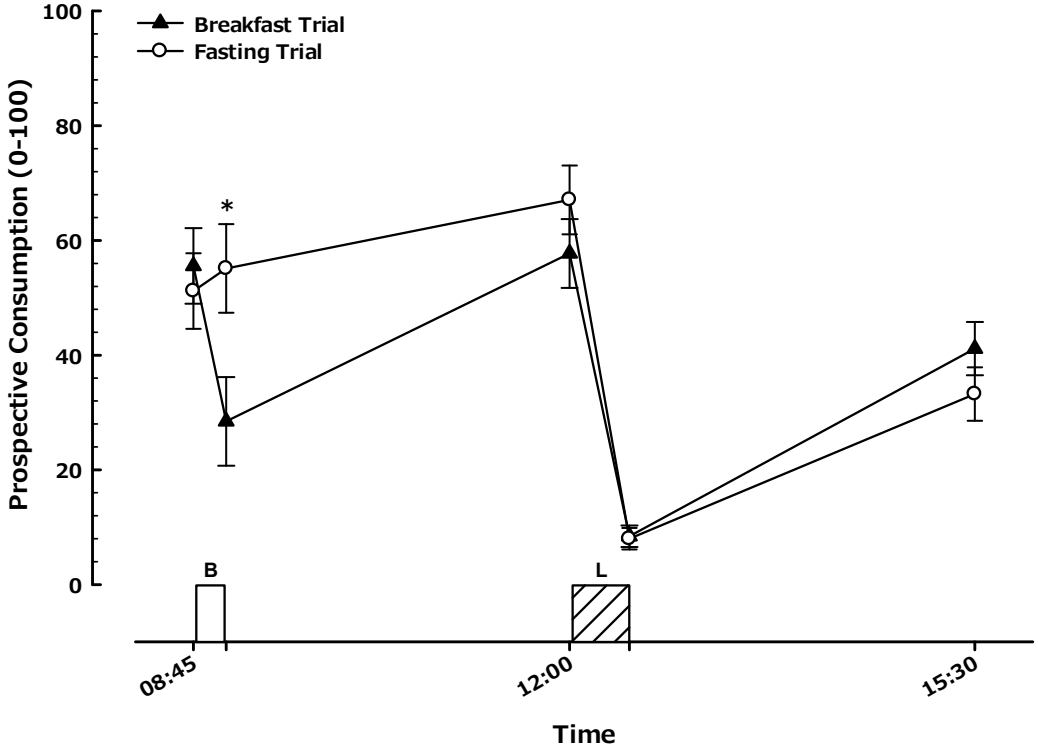

Figure 6.14: Prospective consumption during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial

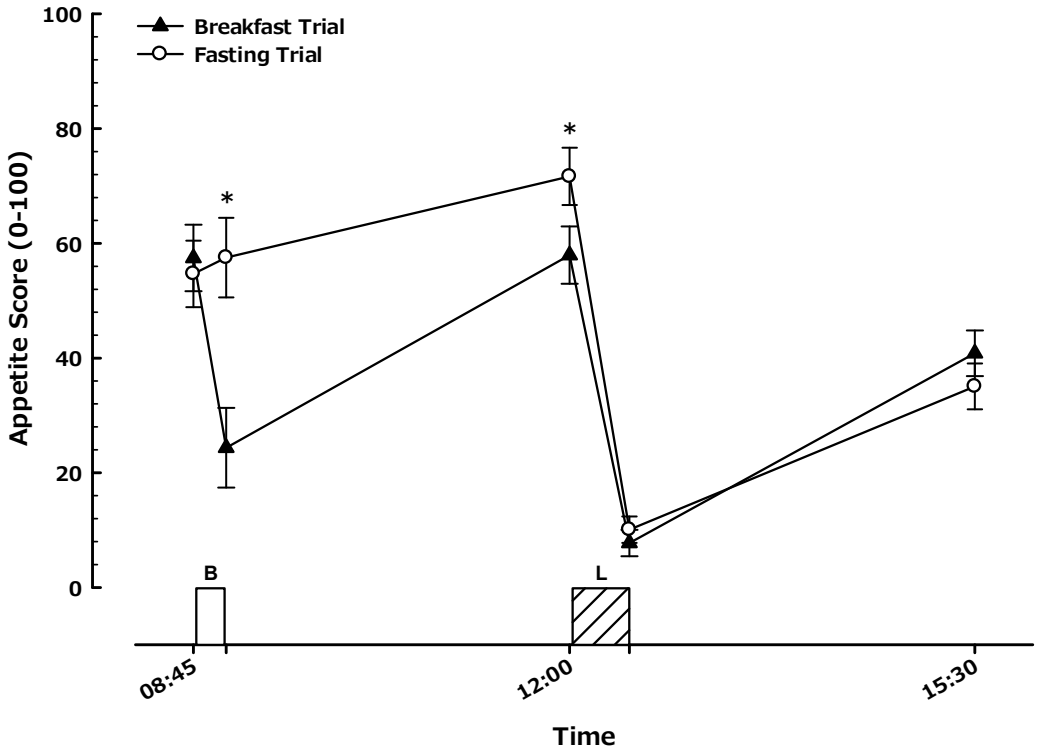

Figure 6.15: Appetite score during trials. B=Breakfast period, in which participants ate a prescribed breakfast during the breakfast trial and rested during the fasting trial. L= *Ad libitum* pasta lunch. * $p < 0.01$ versus corresponding time point in other trial. *Equivalent figures for appetite ratings for lean individuals on pages 108-110*

6.3.10 Subjective Appetite Ratings

Subjective appetite ratings are displayed in Figures 6.11-15. Main effects of time and trial and a time x trial interaction were apparent for desire to eat (all $p < 0.01$). During the morning desire to eat was greater in those fasting than those who ate breakfast both immediately after consumption and prior to lunch (both $p < 0.01$). There was a tendency for greater desire to eat in the breakfast trial at the end of the day (40 ± 20 *vs* 31 ± 24; $p = 0.06$).

For prospective consumption effects of time, trial and a trial x time interaction were apparent (all $p < 0.05$). Following breakfast consumption prospective consumption was reduced and was less than in the fasting trial ($p < 0.01$). There was a tendency for the pre-lunch prospective consumption to be lower in the breakfast trial than the fasting trial (Breakfast, 58 ± 15 *vs* Fasting, 67 ± 20; $p = 0.06$). Lunch consumption resulted in similar post-lunch sensations ($p = 0.7$), however, by the end

of the day there was a tendency for higher prospective consumption in the breakfast (41 ± 19) than fasting trial (33 ± 20; $p = 0.06$).

For hunger there were main effects apparent for time and trial as well as a time x trial interaction (all $p < 0.01$). Hunger was lower throughout the morning during the breakfast trial (both $p < 0.01$). There were no differences in perceived hunger throughout the afternoon between trials (both $p > 0.18$).

Results for fullness indicated main effects of trial, time and a trial x time interaction (all $p < 0.03$). Fullness was increased after breakfast consumption and was greater than in the fasting trial (71 ± 18 *vs* 29 ± 21; $p < 0.05$). Prior to lunch, there was a tendency for greater fullness in the breakfast trial (33 ± 19 *vs* 20 ± 18; $p = 0.06$). Immediately after lunch and at the end of the day, there was no detectable difference in fullness (both $p > 0.1$).

For the composite appetite score calculated there was a main effect of trial, time and a trial x time interaction (all $p < 0.03$). Appetite was reduced following breakfast consumption and although it rebounded to baseline levels prior to lunch, it was still lower than in the fasting trial (both $p < 0.01$). There was no difference in appetite after lunch consumption ($p = 0.15$) but there was a tendency for greater appetite at the end of the testing day in the breakfast trial (41 ± 17 *vs* 35 ± 17; $p = 0.09$).

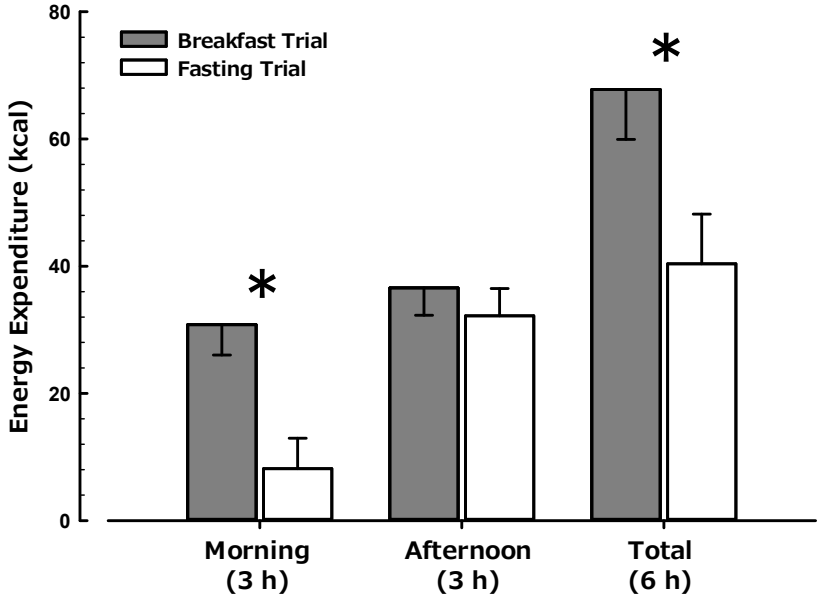

Figure 6.16: Diet induced thermogenesis during trials. The morning and afternoon periods were both of 3 h duration. * $p < 0.01$ versus other trial. *Equivalent figure for lean individuals on page 112*

6.3.11 Diet Induced Thermogenesis

Energy expenditure above rest was greater ($p < 0.01$) in the breakfast (31 ± 15 kcal·3h^{-1}) than fasting (8 ± 13 kcal·3h^{-1}) trial during the morning. Following lunch consumption there was a tendency ($p = 0.08$) for greater energy expenditure in the breakfast trial (37 ± 17 kcal·3h^{-1}) when compared with the fasting trial (32 ± 16 kcal·3h^{-1}). When energy expenditure above rest was combined for the trial as a whole, there was greater energy expenditure in the breakfast than fasting trial (Breakfast, 68 ± 30 kcal·6h^{-1} *vs* Fasting, 40 ± 23 kcal·6h^{-1}; $p < 0.01$).

6.4 Discussion

The current study aimed to characterise the metabolic, hormonal and appetite responses to morning fasting compared with a typical high carbohydrate breakfast in obese individuals. There was no evidence of altered energy intake at an *ad libitum* lunch when participants fasted, resulting in greater net intake in the breakfast trial. Following lunch consumption there was limited suppression of ghrelin in the breakfast trial, but greater PYY and leptin concentrations. Subjective ratings did not indicate greater appetite after lunch consumption in the fasting trial, despite less energy intake over the testing day. These results indicate that obese individuals do not compensate for missed breakfast energy intake at a lunchtime meal and that neither hormonal nor subjective measures of appetite are increased during the afternoon following fasting.

The finding of incomplete energy compensation in a single meal following morning fasting has been reported previously in lean individuals (Levitsky and Pacanowski, 2013) and was also observed in Chapter 3 of this thesis. Previous studies using morning preloads have reported both increased (Astbury et al., 2011) and unaffected lunchtime intake (Gonzalez et al., 2013) following fasting. However, all of these published studies have been completed in lean, young participants. To the best of our knowledge, this is the first study to specifically examine a contrast of acute morning fasting against breakfast consumption in obese individuals. Although it has been suggested that energy intake is increased with breakfast skipping (Farshchi et al., 2005b) there is accumulating evidence from randomised controlled trials that energy intake is either unaffected (Halsey et al., 2012) or lower in individuals who skip breakfast (Reeves et al., 2014). This latter finding of reduced energy intake with omission of breakfast was also replicated in free-living lean individuals in Chapter 4. Therefore, the effects of morning fasting upon total energy intake are yet to be conclusively established. It would seem that any potential compensation for omitted energy intake through breakfast skipping is not strongly manifested through energy intake at the lunchtime meal in obese individuals. However, it is plausible that energy intake in a natural environment would be discrepant from the results obtained in the laboratory due to self-regulation of feeding patterns and food choices. This is also particularly relevant as obese individuals display elevated neural responses to palatable and energy dense foods (Burger and Berner, 2014) and are suggested to be

more susceptible to environmental cues to eat than hormonal regulation (Schachter, 1968; Mela, 2001). Studies investigating free-living energy intake with morning fasting and lab studies utilising buffet designs or permitting volitional feeding frequency are warranted in obese individuals.

The current work replicates our findings in lean individuals that consuming a typical carbohydrate rich breakfast results in blunted ghrelin response to a second meal (i.e a lack of suppression after feeding) despite similar lunch energy intake in this obese group. This finding is also similar to a previous study reporting absence of ghrelin suppression in obese individuals following food intake (English et al., 2002). Similar to the responses observed in lean individuals, this limited suppression of ghrelin may be linked to reduced glucose and insulin responses to the lunchtime meal following breakfast consumption that is putatively due to the second meal effect (Bonuccelli et al., 2009). Whether this effect persists with breakfast and lunchtime meal combinations inducing less pronounced differences in glucose and insulin relative to morning fasting is an interesting area of future study.

Similarly to the lean individuals previously studied using the same design in Chapter 3, leptin and PYY were greater in the afternoon during the breakfast trial. Therefore, in individuals consuming breakfast there were both hormonal responses generally indicative of satiety (PYY/leptin) as well as others that may signal hunger (ghrelin). In contrast to the lean individuals previously studied, and within the context of these contrasting hormonal responses, there was some tentative evidence of increased perceptions of hunger at the end of the day in the breakfast trial. Future work extending laboratory investigations into the evening would provide interesting information as to the time course of these hormones/subjective perception of appetite into the evening and if there are any resultant effects upon energy intake.

Immediately prior to lunch, as would be expected, subjective ratings of appetite indicated greater hunger during the fasting trial. However, despite marked differences in subjective ratings of appetite there was no difference in lunchtime energy intake between the two trials. This is not completely unexpected as the correlation between pre-lunch appetite ratings and *ad libitum* lunch energy intake in lean men has been reported as only in the range of 0.25 to 0.38 indicating a weak to

moderate relationship (Flint et al., 2000) and a further meta-analysis has indicated that sensations of hunger and satiety did not correlate with energy intake in obese individuals (Flint et al., 2007).

There are several plausible explanations for this lack of relationship between pre-lunch appetite ratings and energy intake. Firstly, that the relationship between appetite and intake are disrupted in obese individuals (Flint et al., 2007). Secondly, that subjective appetite measures do not adequately capture the various dimensions of appetite (Mattes et al., 2005). Thirdly, that there is another factor that potentially led to termination of the meal in participants who were still hungry. Meal termination may have arisen due to feelings other than satiation (i.e boredom and reduced liking for the food provided due to the homogeneity of the meal provided) as provision of a variety of foods delays satiation (Hetherington et al., 2006) and promotes greater intake (Rolls et al., 1981b; Spiegel and Stellar, 1990).

The present study shows morning fasting does not result in energy compensation at an *ad libitum* lunch meal compared with consuming a carbohydrate rich breakfast in obese individuals. Breakfast consumption limited the suppression of ghrelin following the lunchtime meal as was also previously observed in lean individuals, with this response possibly mediated through reduced insulin responses to lunch. There was no evidence of increased hunger in the late afternoon when individuals fasted throughout the morning. This work suggests that any compensation for missed energy intake is not apparent at the next meal in obese individuals, although the possibility that this may occur through greater meal/snack frequency and food choices should be investigated in free-living settings.

Chapter 7: Effect of six weeks of daily morning fasting upon components of energy balance and associated health markers in obese individuals

7.1 Introduction

As discussed in Chapter 1, epidemiology has associated infrequent breakfast consumption with increased risk of adiposity (Ma et al., 2003; Horikawa et al., 2011; Purslow et al., 2008; Barton et al., 2005; Kant et al., 1995), diabetes (Mekary et al., 2013; Mekary et al., 2012) and cardiovascular risk (Cahill et al., 2013b; Smith et al., 2010). However, it is also pertinent that breakfast consumers have been found to exhibit other healthful behaviours including consumption of less fat and alcohol (van der Heijden et al., 2007), are more likely to be non-smokers (van der Heijden et al., 2007; Smith et al., 2010) and, crucially, are more physically active (Duval et al., 2008). Therefore, despite numerous studies examining associations between breakfast and health, either the existence or direction of causality cannot be inferred from cross sectional studies. It remains to be established if breakfast is causally linked to health or is instead a marker of a healthy lifestyle.

While previous laboratory studies have contrasted breakfasts of varied quantity or composition, for example by manipulating macronutrient (Clegg and Shafat, 2010), fibre (Hamedani et al., 2009; Kim et al., 2009; Levine et al., 1989; Liljeberg et al., 1999) or energy content (Martin et al., 2000; Hubert et al., 1998) and/or glycaemic index (Rosen et al., 2011; Nilsson et al., 2008). Few studies have contrasted energy intake with a no breakfast condition (Gonzalez et al., 2013; Astbury et al., 2011; Levitsky and Pacanowski, 2013). Two of these studies utilised a preload (i.e a mid-morning snack) and as such were not specifically examining unbroken morning fasting (Gonzalez et al., 2013; Astbury et al., 2011). We have investigated the acute appetite responses to morning fasting *versus* a typical carbohydrate rich breakfast in both lean (Chapter 3) and obese (Chapter 6) individuals. In both cohorts, lunch intake was not increased sufficiently to compensate for the intake at breakfast (i.e net energy intake was greater in the breakfast consumption trial). While these studies provide useful insight into mechanisms of short-term appetite regulation, free-living studies are

required to provide an ecologically valid reflection of how morning fasting can impact upon both energy intake and overall energy balance.

If breakfast consumption can favour a more negative net energy balance, one mechanism may be a reduction in energy intake throughout the rest of the day. However, epidemiological evidence is mixed, with some authors reporting no difference (Song et al., 2005; Wyatt et al., 2002) and others greater intake in those who consume breakfast (Cho et al., 2003; Nicklas et al., 1998). Consistent with the latter, emerging findings from randomised controlled trials in free-living adults indicate greater intake with breakfast consumption (Reeves et al., 2014).

In Chapter 4 of this thesis we have also observed lower free-living energy intake in those fasting during the morning. However, as discussed in Chapter 6, there are several differences between appetite regulation of lean and obese individuals that may affect their responses to a free-living intervention. Therefore, it is important to establish how observed responses in lean individuals undergoing a morning fasting/daily breakfast intervention may differ in obese individuals.

In addition to the potential for reduced energy intake, another key malleable component of energy balance that may respond to breakfast consumption/morning fasting and therefore explain a more negative net energy balance with breakfast consumption is greater energy expenditure. The studies that have contrasted daily breakfast with morning fasting in adults have either not measured energy expenditure (Farshchi et al., 2005b; Schlundt et al., 1992) or have used partial (~8 h) records of physical activity assessed via pedometers and heart rate monitoring (Halsey et al., 2012), which limits the sensitivity of the physical activity measurement undertaken (Butte et al., 2012). The lack of difference between conditions in individuals undertaking both breakfast consumption and morning fasting observed by Halsey and colleagues (2012) in lean individuals contrasts with the findings from Chapter 4 of this thesis; where greater energy expenditure was apparent in those lean individuals assigned the daily breakfast consumption intervention. It therefore remains to be established if physical activity energy expenditure is affected by morning feeding patterns.

In addition to differences between those fasting and consuming daily breakfast in several elements of energy balance in lean individuals, we have also reported the responses of several markers of health during and following the interventions. We found no detrimental metabolic impact of morning fasting, apart from increased afternoon/evening glucose variability from weeks 1 to 6 of the intervention. Prior work by Farshschi and colleagues (2005) reported a negative impact upon fasting lipids and insulin iAUC to a mixed macronutrient test drink of extending the morning fast until 11am in lean women. While we have not observed such pronounced negative implications of morning fasting in lean individuals, studies have yet to determine the effect of a morning fasting intervention upon health markers in free-living obese individuals.

The aim of the current study is to examine the impact of daily morning fasting/breakfast interventions on energy balance and selected markers of health in obese individuals. On the basis of prior published work and our free-living study in lean individuals described in Chapter 4, we hypothesise that both energy intake and physical activity expenditure will be lower in individuals fasting during the morning but that adherence to this regimen will not cause significant deterioration of health markers (i.e insulin sensitivity, fasting lipids).

7.2 Participants and Methods

7.2.1 Participants

A total of 23 healthy, obese men (n = 8) and women (n = 15) aged 25-58 y volunteered to take part in this study. Participants were initially assessed for eligibility based upon the general criteria outlined in Chapter 2. In this study participants were recruited with a body mass index of ≥ 30 $kg \cdot m^{-2}$ and then later classified as obese based upon DEXA-derived fat mass indices of ≥9 $kg \cdot m^{-2}$ (men) and ≥13 $kg \cdot m^{-2}$ (women) (Kelly et al., 2009). Participants were required to report being weight stable (± 2 % body mass fluctuation within past 6 months). Participants adhered to a standard sleep-wake cycle (e.g no shift workers) and did not anticipate any change in lifestyle during the study period. Participants were free of metabolic disorders, with pre-menopausal female participants either menstruating regularly, or following their chosen contraceptive method (i.e pill, implant) for >6 months. These individuals were randomly assigned to one of two intervention groups, either a breakfast consumption group prescribed ≥ 700 kcal by 11:00 daily (at least half of which was consumed within two hours of waking), or a fasting group prescribed abstinence from energy providing nutrients (i.e plain water only) until 12:00 daily. Characteristics of participants in the two groups are presented in Table 7.1.

Written informed consent was obtained from all participants, with the Ethical Approval for the study obtained from the NHS Bristol Research Ethics Committee. The study is registered with Current Controlled Trials [ISRCTN31521726].

Table 7.1: Participant characteristics

Characteristic		Breakfast Group	Fasting Group
n		11	12
Age (y)		44 (10)	44 (10)
Body Mass (kg)		103.9 (24.0)	92.4 (11.2)
Body Mass Index (kg/m^2)		35.4 (6.1)	32.0 (2.2)
Fat Mass Index (kg/m^2)*	**All**	14.8 (5.0)	12.0 (2.3)
	Female	16.9 (4.5)	13.5 (1.8)
	Male	9.9 (1.4)	9.8 (0.8)
Habitual Breakfast Consumers (*n*)		7	7
Female (*n*)		7	8

*Fat mass index calculated as DEXA derived total fat mass divided by height squared. Values represent mean with (SD)

7.2.2 Study Methodology

The study design, experimental protocols and approach to statistical analysis for this investigation were identical to those outlined in Chapter 4 of this thesis. The only difference from procedures employed for lean individuals described in Chapters 2 and 4 was for the analysis of physical activity data. Due to a tendency for reduced wear time and heart rate data quality in obese participants, the criteria for Actiheart® monitoring were relaxed slightly to maximise included participant numbers without significantly compromising data quality. As such, a valid day of measurement for obese individuals was considered as 85% wear time for a day and no more than 30% comprising "recovered data". This contrasts with corresponding criteria of 90% wear time and 22.5% maximum for "recovered data" in lean individuals. The impact of these changes upon the data obtained is presented in further detail in Appendix 2. Other inclusion criteria for physical activity monitoring data remained the same as lean individuals as described in Chapter 2.

7.3 Results

7.3.1 Body Composition

Body mass increased across both groups in response to the intervention (F = 6.293, p = 0.02). There was no difference between groups (F = 2.23, p = 0.15) or an interaction effect (F = 2.29, p = 0.15). Pre-planned contrasts for change in body mass within each group revealed significantly increased body mass in the breakfast group (0.96 kg ± 1.10 kg; p = 0.02) but not in the fasting group (0.24 ± 1.13 kg; p = 0.5).

Table 7.2: Anthropometric measures

Measure	*Breakfast*		*Fasting*	
	Pre	Change	Pre	Change
Total Mass (kg)	103.9 (24.0)	1.0 (0.2, 1.7)	92.4 (11.2)	0.2 (-0.5, 1.0)
Lean Mass (kg)	52.5 (7.0)	0.2 (-1.7, 2.1)	54.7 (11.0)	-0.2 (-1.3, 0.8)
Fat Mass (kg)	41.8 (12.8)	0.5 (-1.4, 2.5)	34.1 (3.5)	0.6 (-0.4, 1.5)
Waist:Hip ratio	0.87 (0.10)	0.01 (-0.01,0.03)	0.91 (0.07)	-0.01 (-0.03, 0.00)
Sagittal Abdominal Diameter (cm)	26.4 (3.2)	0.0 (-0.4, 0.4)	25.2 (2.0)	-0.4 (-1.0, 0.2)

Values represent mean with (SD) and change scores from baseline with (95 % CI).
Equivalent table for lean individuals on page 126

Lean mass estimated by DEXA was not different between groups, over time and there was no interaction effect of time x group (all p > 0.6). Fat mass did not differ over time (F = 1.38, p = 0.25), and groups did not respond differently to the intervention (F = 0.00, p = 0.98). There was a tendency for a difference between groups, with greater fat mass in the breakfast group (F = 3.93, p = 0.06). It should be noted that one individual in the breakfast group could not be scanned due to their size and body mass (167 kg) exceeding the maximum capacity of the scanner, so it is likely that this group difference would be greater were data available for this individual. Measures of central adiposity were not different between groups, or over the course of the intervention (all p > 0.2). Groups did not respond differently to the intervention for any measure (both p > 0.1), apart from a tendency for an interaction effect for Waist:Hip ratio (F = 3.166, p = 0.09).

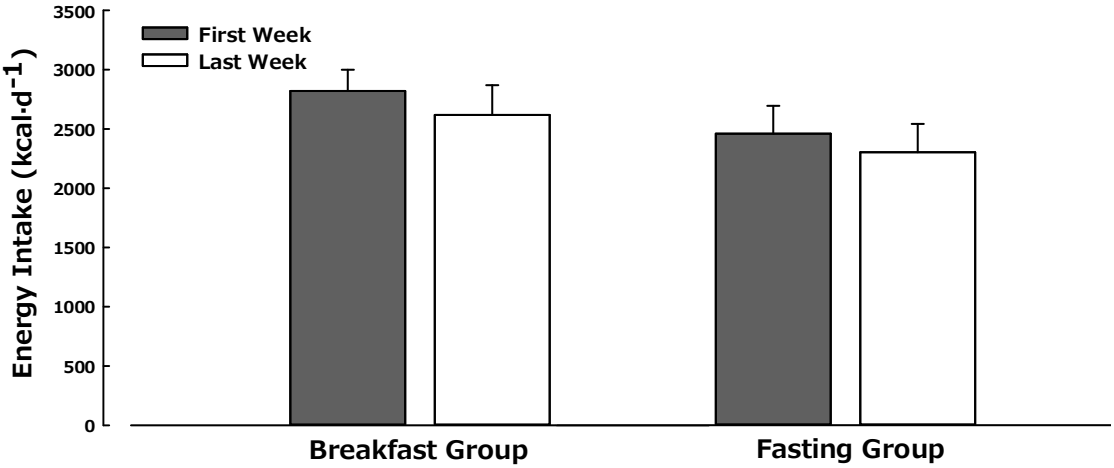

Figure 7.2: Reported energy intake during the first and last week of the intervention.
Equivalent figure for lean individuals on page 127

7.3.2 Energy Intake

Reported daily energy intake during the intervention was not different between groups (F = 1.173, p = 0.29), with the mean energy intake of the two weeks of monitoring 2719 ± 683 kcal·d^{-1} in the breakfast group and 2382 ± 234 kcal·d^{-1} in the fasting group. There was a main effect of time for reported energy intake from the first to the last week of the intervention (F = 4.886, p = 0.04), this decrease was -202 kcal·d^{-1} (95% CI,-526, 122) in the breakfast group and -156 kcal·d^{-1} (95% CI, -315, 3) in the fasting group.

There were no differences (all p > 0.1) between groups for percentage macronutrient composition for the whole day (Table 7.3) or total intake of macronutrients (Figure 7.3). There were main effects of time for total and saturated fat intake (both p < 0.05), and a tendency for a time x group interaction (p = 0.06) for total fat intake.

Table 7.3: Percentage composition of diets during intervention

	Breakfast Group						**Fasting Group**	
	First Week			**Last Week**			**First Week**	**Last Week**
	Pre 12:00	**Post 12:00**	**Total**	**Pre 12:00**	**Post 12:00**	**Total**	**Post 12:00**	**Post 12:00**
Energy Intake ($kcal \cdot d^{-1}$)	885 (197)	1934 (538)	2820 (595)	891 (214)	1727 (742)	2618 (833)	2459 (779)	2303 (792)
Protein %	15.7 (4.6)	15.8 (3.2)	15.7 (2.4)	14.4 (3.4)	17.1 (3.9)	16.1 (3.4)	14.3 (2.2)	15.2 (3.2)
Carbohydrate %	51.2 (14.9)	41.6 (4.4)	44.3 (6.2)	50.3 (14.5)	44.0 (5.7)	45.5 (7.6)	44.0 (8.6)	43.6 (7.5)
Fat %	33.1 (11.9)	38.7 (4.4)	37.4 (5.5)	35.3 (12.0)	34.4 (5.5)	35.2 (6.4)	35.0 (7.2)	35.8 (6.0)
Alcohol %	n/a	3.9 (4.2)	2.7 (2.8)	n/a	4.6 (4.7)	3.2 (3.4)	6.7 (8.4)	5.4 (6.3)

Values represent mean with (SD). *Equivalent table for lean individuals on page 129*

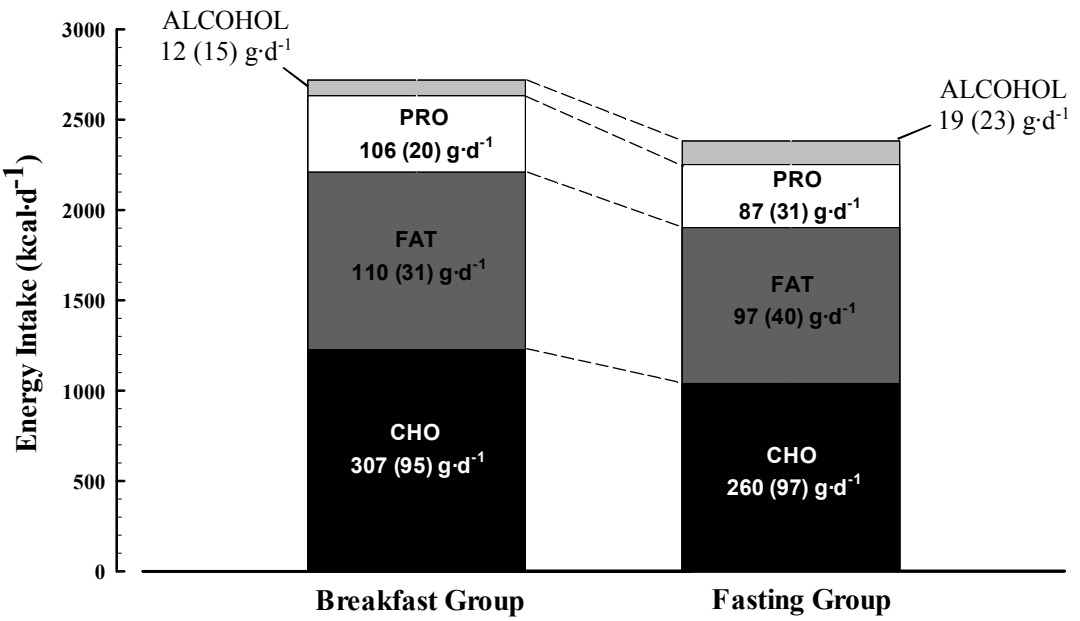

Figure 7.3: Daily macronutrient intake during the intervention. Figures on stack represent mean intake, with figures in brackets representing standard deviation. *Equivalent figure for lean individuals on page 130*

When comparing pre- and post-12:00 percentage macronutrient composition in the breakfast group, there were no differences (all $p \geq 0.1$) between any of the macronutrients. However, there was a greater proportion of energy intake from sugars prior to 12:00 relative to post 12:00 (23 ± 8 % ± *vs* 16 ± 4%, $p = 0.02$).

Once both groups were permitted unrestricted feeding (i.e 12:00 onwards) there was a tendency towards greater energy intake in the fasting group (2381 ± 777 kcal·d^{-1}) relative to the breakfast group (1831 ± 612 kcal·d^{-1}; $p = 0.08$). There was greater intake of sugars in the fasting group after 12:00 (108 ± 45 g *vs* 75 ± 27 g; $p = 0.05$) but no other differences between groups were apparent for macronutrient intakes after 12:00 (all $p > 0.1$). There was reduced reported intake ($F = 6.05$, $p = 0.02$) in the last week relative to the first week of recording. For total fat and saturated fat intake there was a main effect of time, with reductions across both groups (both $p < 0.03$). A trial x group interaction was apparent for total fat intake ($F = 4.58$, $p = 0.05$), with a significant reduction in the breakfast group from the first (85 ± 28 g) to last week (66 ± 26; $p < 0.01$).

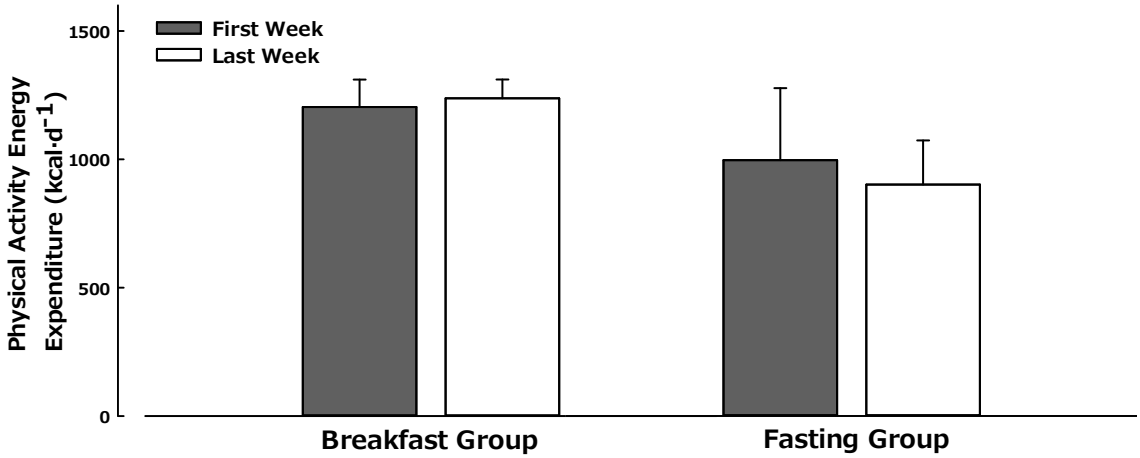

Figure 7.4: Physical activity energy expenditure. *Equivalent figure for lean individuals on page 132*

7.3.4 Physical Activity Energy Expenditure

Daily energy expenditure between the two groups was not different (Breakfast Group, 1221 ± 261 kcal·d^{-1} *vs* Fasting Group, 949 ± 709 kcal·d^{-1}; F = 1.17, p = 0.29). There was no difference in energy expenditure between the two weeks of physical activity monitoring, and the groups did not respond differently between the first and last week of monitoring (both $p > 0.3$). There was no difference between the two weeks of measurement, with both groups stable within 100 kcal·d^{-1} between weeks and no time x group interaction (F = 0.83, p = 0.37).

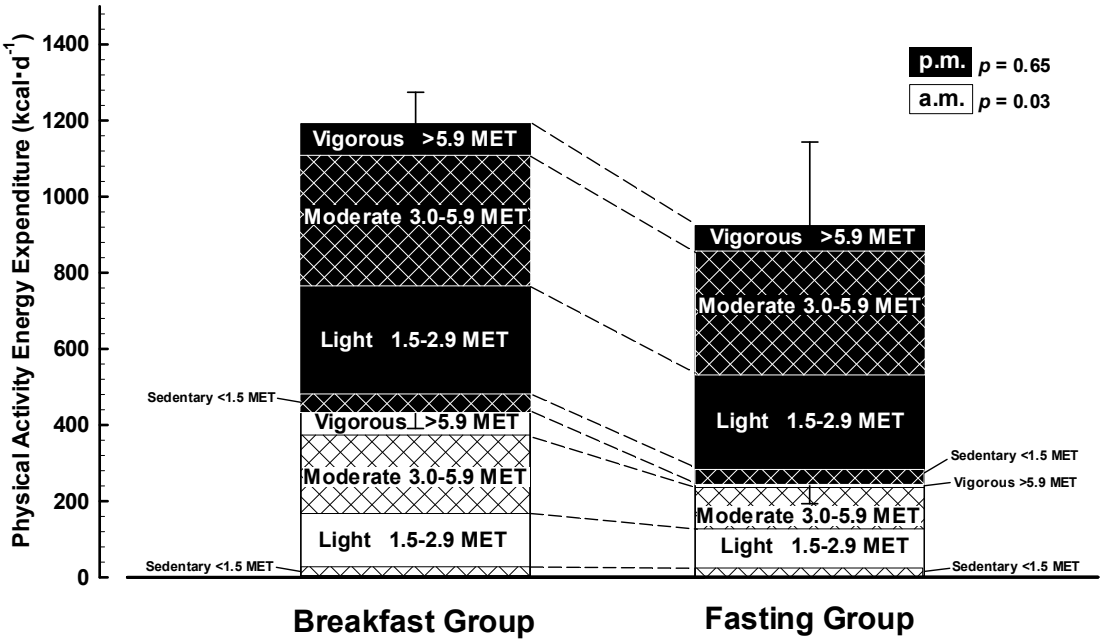

Figure 7.5: Physical activity energy expenditure split by time of day and intensity. *Equivalent figure for lean individuals on page 134*

Analysis of the timing/intensity of energy expenditure is displayed in Figure 7.5 and Table 7.4. This indicated that energy expenditure prior to 12:00 was greater in the breakfast group (435 ± 132 kcal·d^{-1}) than the fasting group (247 ± 171 kcal·d^{-1}; p = 0.03). After 12:00 there was no significant difference in energy expenditure between the two groups (Breakfast Group, 756 ± 135 kcal·d^{-1} *vs* Fasting Group, 676 ± 540 kcal·d^{-1}; p = 0.66). Activity partitioned by intensity according to metabolic equivalents was not different between groups, either for the day considered as a whole or for the pre-, or post-12:00 periods (all $p > 0.1$).

Table 7.4: Physical activity energy expenditure split by intensity and time of day

	Breakfast Group			Fasting Group		
Energy Expenditure (kcal·d^{-1})	**AM**	**PM**	**24h**	**AM**	**PM**	**24h**
Sedentary (<1.5 MET)	24 (7)	48 (11)	72 (17)	26 (7)	41 (12)	67 (18)
Light (1.5-2.9 MET)	146 (30)	284 (55)	430 (79)	103 (57)	247 (100)	350 (146)
Moderate (3.0-5.9 MET)	208 (82)	346 (116)	554 (193)	109 (99)	327 (332)	435 (424)
Vigorous (6.0-10.1 MET)	50 (68)	75 (56)	125 (115)	9 (16)	39 (72)	49 (85)
Very Vigorous (≥10.2 MET)	8 (16)	3 (4)	11 (20)	1 (2)	22 (46)	23 (47)
Total	435 (132)	756 (457)	1191 (249)	247 (171)*	676 (540)	923 (697)

Values represent mean with (SD). *Equivalent table for lean individuals on page 135*
* Denotes significantly lower in fasting than breakfast group ($p < 0.05$)

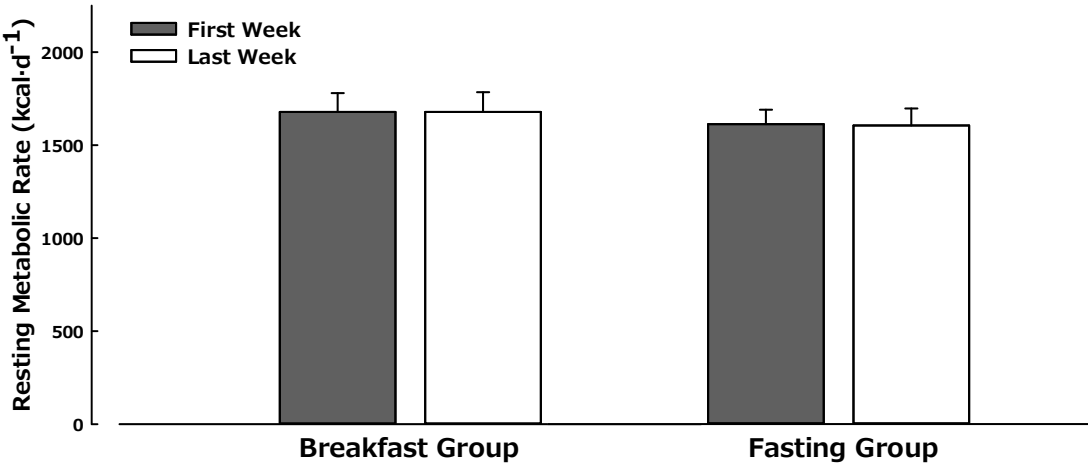

Figure 7.6: Resting metabolic rate before and after the intervention. *Equivalent figure for lean individuals on page 136*

7.3.5 Resting Metabolic Rate

There were no main effects of time or group (both $p > 0.6$) for resting metabolic rate. Resting metabolic rate was 1613 ± 77 kcal·d^{-1} and 1679 ± 101 kcal·d^{-1} for the fasting and breakfast groups, respectively, prior to the intervention. Following the intervention, RMR was stable in both groups (Fasting Group, 1605 ± 91 kcal·d^{-1} *vs* Breakfast Group 1679 ± 106 kcal·d^{-1}) such that it was within 8 kcal from prior to the interventions in both groups, with no difference in response to the intervention ($F = 0.04$, $p = 0.8$, Figure 7.6).

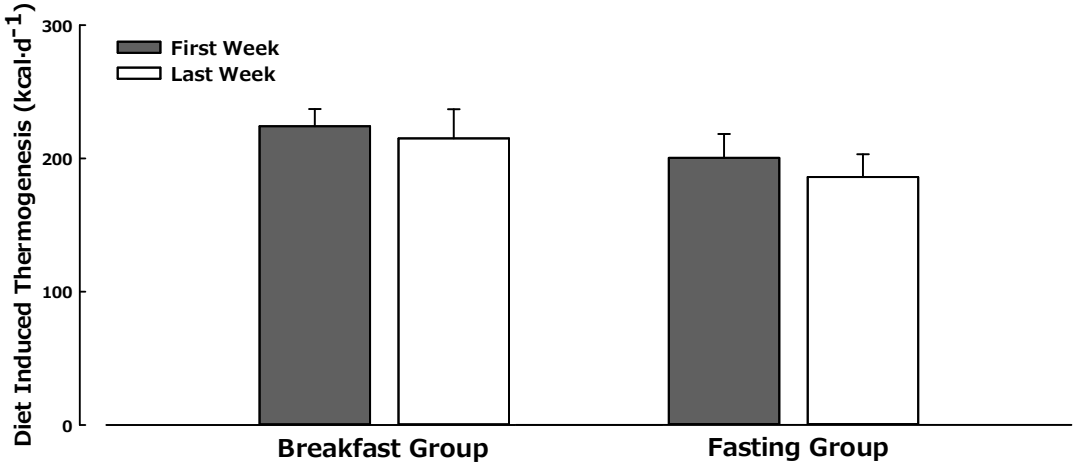

Figure 7.7: Diet induced thermogenesis during the intervention. *Equivalent figure for lean individuals on page 137*

7.3.6 Diet Induced Thermogenesis

Based on the established thermogenic effect of ingested macronutrients, diet induced thermogenesis based on reported energy intake from food diaries was not different between groups ($F = 1.22$, $p = 0.28$), with no effect of time ($F = 2.36$, $p = 0.14$) or any interaction ($F = 0.12$, $p = 0.73$). Daily diet induced thermogenesis was 200 ± 60 kcal·d^{-1} in the fasting group, relative to 224 ± 43 kcal·d^{-1} in the breakfast group, during the first week. In the final week of observation, diet induced thermogenesis was 186 ± 57 kcal·d^{-1} in the fasting group relative to 215 ± 72 kcal·d^{-1} in the breakfast group.

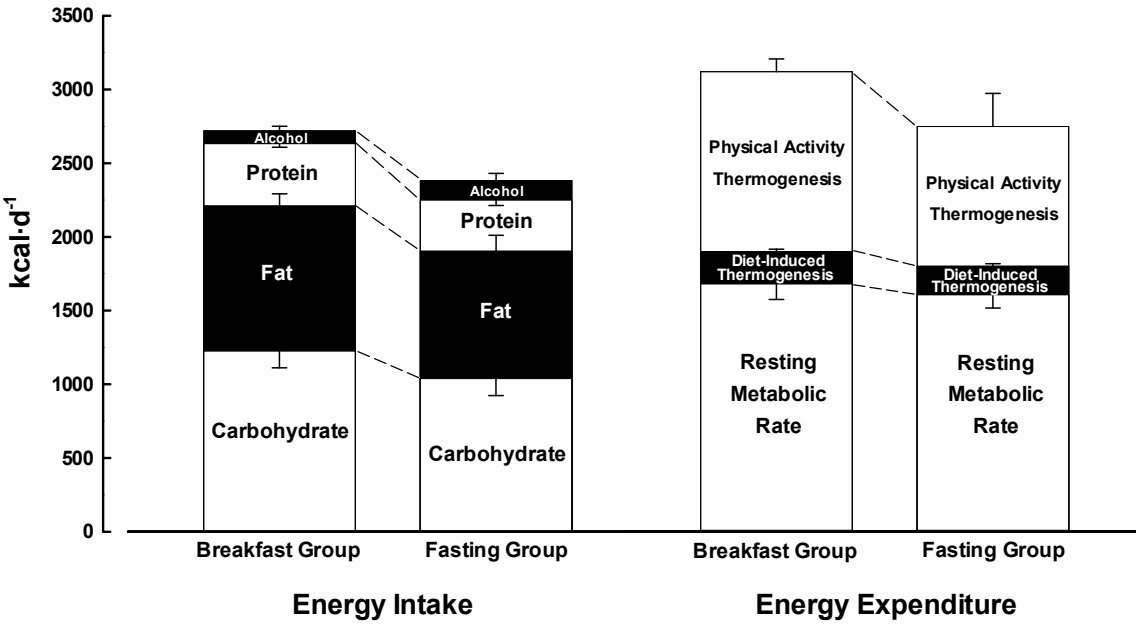

Figure 7.8: Energy Balance Summary. *Equivalent figure for lean individuals on page 138*

7.3.7 Oral Glucose Tolerance Test

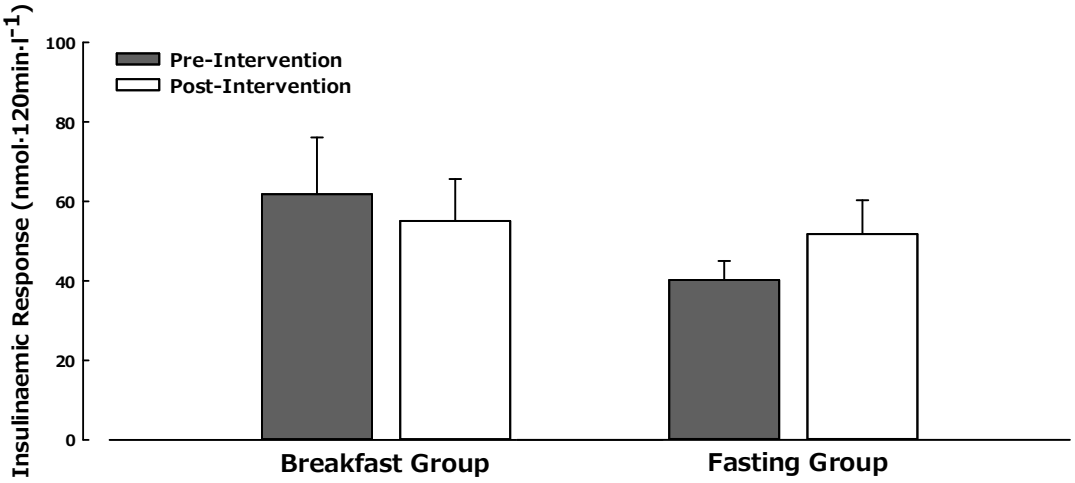

Figure 7.9: Insulinaemic response to an Oral Glucose Tolerance Test. *Equivalent figure for lean individuals on page 139*

Insulin concentrations varied over the course of the OGTT ($F = 16.24$, $p < 0.01$) and this response over time varied from pre- to post-intervention ($F = 3.732$, $p = 0.02$). There were no other main effects or interactions (all $p > 0.3$) apart from a tendency for a trial x group interaction ($F = 3.84$, $p = 0.07$). Insulin incremental AUC was not different between groups or trials (both $p > 0.3$), but there was a significant trial x group interaction ($F = 4.72$, $p = 0.05$, Figure 7.9). The individual contrasts of the insulinaemic response to the OGTT from pre- to post-intervention within each group indicated no significant differences in either group (both $p > 0.1$).

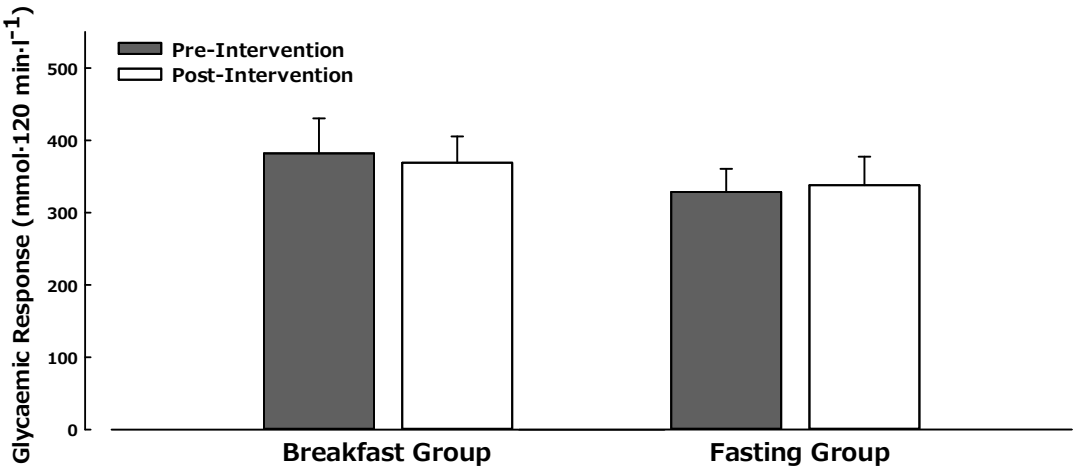

Figure 7.10: Glycaemic response to an Oral Glucose Tolerance Test. *Equivalent figure for lean individuals on page 139*

Glucose concentrations varied over the course of the OGTT ($F = 34.55$, $p < 0.01$) and this response over time varied from pre- to post-intervention ($F = 4.03$, $p = 0.01$). No other main effects or interactions were apparent for glucose concentrations during the OGTT (all $p > 0.17$). Glucose incremental AUC was not different over time, between groups and the response to the intervention was not different between groups (all $p > 0.3$; Figure 7.10).

Table 7.5: Insulin sensitivity measures

Measure		*Breakfast Group*		*Fasting Group*	
		Pre	Change	Pre	Change
Fasting Insulin	($pmol.l^{-1}$)	63 (35)	2 (-21, 25)	54 (16)	-4 (-16, 9)
Peak Insulin	($pmol.l^{-1}$)	872 (555)	-54 (-233, 124)	694 (285)	101 (-99, 303)
Fasting Glucose	($mmol.l^{-1}$)	5.30 (0.29)	0.08 (-0.12, 0.28)	5.56 (0.37)	0.09 (-0.20, 0.38)
Peak Glucose	($mmol.l^{-1}$)	10.09 (1.40)	0.30 (-0.53, 1.14)	10.15 (1.00)	0.68 (-0.08, 1.44)
HOMA-IR		2.46 (1.31)	0.18 (-0.84, 1.19)	2.24 (0.72)	-0.13 (-0.71, 0.45)
C-ISI		3.78 (2.05)	0.05 (-0.59, 0.70)	3.81 (1.09)	-0.05 (-1.08, 0.97)

Values represent mean with (SD) and change scores from baseline with (95 % CI).
Equivalent table for lean individuals on page 140

7.3.8 Insulin Sensitivity Measures

Insulin sensitivity measures calculated from the OGTT are displayed in Table 7.5. Fasting glucose and insulin did not respond over time (both $p > 0.3$), with no evidence of a time x group interaction (both $p > 0.6$). There was no difference in fasting insulin between groups ($p = 0.27$) but a difference between fasting glucose concentrations was apparent between groups ($F = 4.44$, $p = 0.05$). There were no main effects or interactions for peak glucose or insulin concentrations (all $p > 0.2$) apart from a tendency for increased peak glucose over time in both groups ($F = 3.90$, $p = 0.06$). Both Matsuda and HOMA indices were not different over time or between groups (all $p > 0.3$), with no evidence of an interaction (both $p > 0.5$).

Table 7.6: Fasting lipids and inflammatory markers

Measure	*Breakfast Group*		*Fasting Group*	
	Pre	Change	Pre	Change
Total Cholesterol ($mmol.l^{-1}$)	5.78 (0.79)	0.14 (-0.11, 0.39)	4.96 (0.90)	0.25 (0.00, 0.49)
HDL Cholesterol ($mmol.l^{-1}$)	1.25 (0.24)	0.03 (-0.06, 0.11)	1.26 (0.27)	-0.01 (-0.16, 0.14)
LDL Cholesterol ($mmol.l^{-1}$)	3.91 (0.98)	0.15 (-0.07, 0.36)	3.07 (0.71)	0.27 (0.01, 0.53)
Total:HDL Cholesterol	4.69 (0.48)	0.03 (-0.18, 0.24)	4.00 (0.70)	0.19 (-0.29, 0.66)
Triglycerides ($mmol.l^{-1}$)	1.87 (1.11)	-0.08 (-0.43, 0.27)	1.38 (0.64)	-0.03 (-0.24, 0.19)
NEFA ($mmol.l^{-1}$)	0.55 (0.27)	-0.13 (-0.3, 0.04)	0.45 (0.21)	-0.00 (-0.11, 0.11)
CRP ($mg.l^{-1}$)	3.10 (2.05)	-0.05 (-0.89, 0.80)	2.19 (1.03)	0.11 (-0.87, 1.08)

Values represent mean with (SD) and change scores from baseline with (95 % CI).
Equivalent table for lean individuals on page 141

7.3.9 Cardiovascular Disease Risk Factors

None of the cardiovascular disease risk factors presented responded differently between groups to the intervention (all $p > 0.13$). There was an increase in total and LDL cholesterol concentrations in both groups from pre- to post-intervention (both $p < 0.03$). Both LDL concentrations and the Total:HDL ratio were greater in the breakfast group (both $p \leq 0.04$), with a tendency for greater total cholesterol concentrations in the breakfast group ($p = 0.07$). No other risk factors differed over time or between groups (all $p > 0.13$).

7.4 Discussion

The current study has examined all elements of energy balance, as well as metabolic control and selected markers of cardiovascular disease risk in response to both daily morning fasting and breakfast consumption for 6 weeks in healthy obese individuals. There was no difference in the overall energy intake reported in the two intervention groups, indicative of dietary compensation (i.e eating more) after 12:00 in those that had fasted during the morning. Physical activity thermogenesis was lower in those fasting prior to 12:00 but was not different over the course of the day as a whole. Resting metabolic rate was stable in response to both interventions and blood lipids and CRP responses to the intervention were not different between the two groups. However, insulinaemic response to an OGTT displayed divergent responses to the interventions with a decrease in those consuming breakfast relative to an increase in those extending their fast. The current study establishes that in free-living obese adults, both breakfast and fasting result in similar overall energy intake and expenditure, with no clear negative impact of daily morning fasting.

Energy intake in those who do not consume breakfast has been reported in cross sectional studies to be both similar to breakfast consumers (Song et al., 2005; Wyatt et al., 2002) and lower than those that consume breakfast (Cho et al., 2003; Nicklas et al., 1998). Experimental evidence in individuals omitting breakfast and not prescribed weight loss has also produced conflicting results, with greater (Farshchi et al., 2005b), similar (Halsey et al., 2012) and lower energy intake (Reeves et al., 2014) in those missing breakfast daily. In our previous study described in Chapter 4, lean individuals undertook the same experimental manipulation as this study. It was found that individuals assigned to the fasting condition had lower energy intake than those consuming breakfast, with minimal dietary compensation throughout the rest of the day.

When attempting to reconcile the present results with those in the extant literature, it is possible that differences in prescriptiveness of the breakfast intervention and weight status may account for some of the discrepancy in findings. Firstly, two of the studies above (Halsey/Reeves) allowed *ad libitum* intake of breakfast and foods throughout the rest of the day, whereas the work of Farshchi prescribed identical foods consumed at 08:00 in the breakfast condition or delayed until 11:00 in the no breakfast

condition and participants then followed scheduled meal patterns. In the present work, individuals in the breakfast group were required to reach a minimum intake of 700 kcal by 11:00 in the breakfast condition. The impact of differing energy intake at breakfast during free-living is not well investigated, although work by Martin and colleagues (2000) has established that free-living total energy intake in young lean men was greater when consuming 700 kcal *versus* a 100 kcal breakfast. This is similar to our findings in lean individuals and may indicate a modifying effect of weight status upon energy intake during breakfast/morning fasting interventions as there was no difference in energy intake between the two experimental groups in these obese individuals.

Reeves and colleagues (2014) have found energy intake is lower with morning fasting when comparing a mixture of lean and overweight/obese individuals who both ate and skipped breakfast for a week. However, this was a pooled effect and the difference in obese individuals (~60 kcal) was much less pronounced than that in lean individuals (~265 kcal). This potentially indicates that, similar to the current work, obese individuals display greater compensation for omitted calories in the morning than lean counterparts. Reasons for greater reported dietary compensation in obese than lean individuals are not immediately apparent, although it should be taken into consideration that underreporting of energy intake is greater in obese individuals (Schoeller, 1995). Despite this, there is no reason to suspect that underreporting should occur to a greater extent in either experimental group (De Castro, 1994b).

In the previous chapter we established that at an *ad libitum* lunch following morning fasting there was no difference in energy intake relative to a breakfast consumption condition. Therefore, it appears that while appetite and lunchtime energy intake is unaffected within a controlled laboratory environment in obese individuals following morning fasting, the response in a free-living setting is markedly different. This finding is in contrast with the observed consistency of effects in lean individuals in the two settings investigated (i.e some evidence of dietary compensation but relatively minimal to the "missed" energy intake through breakfast in both the laboratory and free-living setting). This suggests that external cues may have a more potent effect on obese individuals (Schachter, 1968; Mela, 2001) and that dietary compensation is cumulative throughout the day and not limited to single feeding

occasions. Future analysis of the current data set for the frequency/nature of feeding occasions and distribution of energy intake throughout the day will provide further insight into the dietary compensation stimulated by morning fasting.

Total physical activity thermogenesis was not different between those consuming breakfast and those extending their fast. This is in contrast to our previous findings in lean individuals presented in Chapter 4 where an overall difference between groups for daily physical activity energy expenditure was apparent. This finding is consistent with the lack of difference in energy intake in obese individuals. Unlike the lean group where substantive differences in energy intake and physical activity energy expenditure (both lower in those that fasted) occurred, the impact of the daily fasting intervention appears less marked on components of energy balance in obese individuals. However, some similarity between the lean and obese participants is evident as the fasting obese participants displayed lower physical activity energy expenditure prior to 12:00. This finding reinforces that physical activity energy expenditure is specifically most affected during the period in which energy intake is restricted.

While it might be suggested that this could be an artefact of elevated heart rate due to feeding and not representative of actual physical activity, it should be considered that in response to feeding of 480 kcal mixed food, heart rate elevations of ~5 bpm have been reported in lean and obese women (Matsumoto et al., 2001). Within the context of our choice of measurement device (i.e combined heart rate/accelerometry), in the absence of movement, the weighting of energy expenditure would be 90 % from accelerometry counts and 10 % from heart rate unless heart rate is substantially above rest (far beyond the magnitude induced with feeding as described above, in which case the split is 50:50 between accelerometry and HR). Therefore any potential impact of elevated heart rate solely due to feeding would be minimised. Additionally, in the groups observed, reported energy intake was substantially greater (~550 kcal) after 12:00 in the fasting group but the energy expenditure measured during this period was less than the breakfast group, indicating that energy expenditure measured using this device is not predicated on increased heart rate from feeding.

The specific reasons for lesser physical activity expenditure during the morning when fasting are not immediately apparent, but may be related to perceptions of lethargy/expectations relating to physical activity readiness with fasting. In future it would be instructive to utilise subjective markers of energy throughout the day (e.g the subjective vitality scale (Ryan and Frederick, 1997)) to establish if individuals feel differing levels of energy throughout the day with breakfast and fasting regimens.

Blood lipids were not differently affected by either intervention in this cohort, in opposition to the previous findings of Farshchi et al (2005) who report increased LDL and total cholesterol concentrations after a 2 week breakfast omission intervention in obese women. The previous authors had suggested that this may be due to reduced stimulation of hydroxyl methyl glutaryl-Co-A reductase by insulin as they also reported lower insulinaemic response to a mixed meal challenge following breakfast consumption.

In the present study we have found that similar to the aforementioned work, despite composite measures of insulin sensitivity (i.e HOMA-IR, Matsuda C-ISI) being unchanged there was an interaction for insulin iAUC in response to the OGTT. Although this potentially indicates reduced insulin sensitivity in those that fasted, neither group displayed significantly different insulin iAUC from pre- to post-intervention (both $p > 0.1$). Whilst there was no difference between groups at baseline, there were particularly high values for iAUC in the breakfast group at baseline such that this interaction may partly be influenced by some regression to the mean in this group. Additionally, there was a tendency for the pre-intervention iAUC in the breakfast group to be non-normally distributed ($p = 0.06$), although this should be mitigated as ANOVA are generally robust to such non-normality (Maxwell and Delaney, 1990). It is therefore prudent to consider these findings with some caution as various other measures of insulin sensitivity did not demonstrate similar responsiveness to the interventions. Future work examining the effects of meal ingestion upon glycaemia and insulinaemia following similar interventions may provide confirmatory evidence of these findings.

Randomisation led to two groups that were mostly similar for anthropometric and metabolic measures prior to the intervention. However, it should be acknowledged that whilst the body mass of the groups was not significantly different, the breakfast

group was heavier but with twice the standard deviation for this measure than the fasting group. This was particularly attributable to one extreme outlier for body mass in the breakfast group (167 kg, 2.6 SD above the mean for the group). As this individual did not fulfil any of our stated exclusion criteria at enrolment, we included him in the study and in the analysis. For the majority of pre- and post- measures this individual will act as their own control, so any impact on findings will be accounted for.

However, some salient points should be addressed with regards to their impact upon outcomes measured solely during the intervention and compared between groups (energy intake, physical activity energy expenditure). Importantly, considering the potential for the unusually high body mass of this individual in the breakfast group to affect their energy balance, it should be noted that this individual did not contribute to the physical activity energy expenditure (due to low wear time/lack of heart rate data) in the breakfast group. Therefore, the higher activity levels observed in the morning in the breakfast group are not skewed in the positive direction by this individual with exceptional body mass. Conversely, overall intake was not greater in the breakfast group despite this individual having an intake proportionate to his mass (2.8 SD above the mean for the breakfast group). As a result, the absence of a difference between groups would be even clearer with his exclusion.

The only variable that would be changed with exclusion of this individual is energy intake after 12:00, which would be 716 kcal less (p = 0.01) than the fasting group with removal of this individual. This is compared with an intake that is 550 kcal (p = 0.08) less than the fasting group with this individual included. Whilst the statistical implications would therefore be different for this one variable, the overall conclusion would remain unchanged; that morning fasting and breakfast consumption results in no difference in overall daily energy intake due to some energy compensation from 12:00 onwards. The relative presence or absence of this individual therefore does not alter the overall interpretation of this study for any variable. Despite this, considering the extreme variability possible in obese populations, future work should either employ an upper limit for adiposity, or potentially employ minimisation to facilitate matching of experimental groups on baseline characteristics (Altman and Bland, 2005).

Body mass change was not a key outcome in this study due to the intervention duration of 6 weeks not necessarily being long enough for substantial changes in body mass without a prescribed energy deficit. However, in the current investigation whilst there was no interaction for body mass changes, some relevant conclusions can still be drawn. Considering the prevalent public health message that breakfast consumption facilitates weight management (Brown et al., 2013) it is contradictory that in this investigation 10 of the 11 individuals in the breakfast group gained weight (compared with 6 of 12 in the fasting group) as a result of the intervention (1.0 kg, 95% CI, 0.2, 1.7). In a normal free-living setting without a specific aim of weight loss, breakfast consumption does not appear to confer any benefits for weight management in obese individuals. This may be a product of the nature of the intervention as we specified an energy intake target for breakfast consumers but without prescribing specific food types or any other dietary restrictions. In a large 16 week intervention in individuals interested in weight loss that utilised healthy dietary advice combined with instructions for daily breakfast consumption or fasting until 11:00, there was no difference in weight loss between the breakfast consumers or skippers (Dhurandhar et al., 2014a). Therefore, in relatively natural settings without high levels of dietary prescription, it appears that there is no additional benefit of breakfast consumption for weight management whether attempting to lose weight or without a specific weight loss objective. This study indicates that caution should be exercised in recommendations for breakfast consumption in obese individuals for the specific purpose of weight loss. However, it cannot yet be established if specific breakfast compositions may be necessary to facilitate better weight management.

In summary, we conclude that in obese individuals neither overall energy intake nor physical activity is different in those fasting during the morning or consuming a daily breakfast for 6 weeks, although differences in distribution throughout the day were apparent with lower physical activity during the morning in those fasting. Resting metabolic rate and blood lipids were not differently affected by either intervention but there was some indication of differing effects on insulin sensitivity due to the interventions. Daily morning fasting does not cause weight gain relative to self-selected breakfast in obese individuals.

Chapter 8: Morning fasting for 6 weeks does not cause increased appetite, acute energy intake or negative metabolic consequences to feeding relative to daily breakfast consumption in obese adults

8.1 Introduction

We have previously investigated the effect of chronic assignment (6 weeks) to a breakfast consumption/morning fasting regime upon acute (i.e within a day) appetite regulation in lean individuals in Chapter 5. As discussed in Chapter 6 there are several differences in hormonal regulation of appetite and metabolic responses to feeding in obese individuals in comparison with lean individuals. These include delayed satiation (Delgado-Aros et al., 2004), reduced concentrations of satiety hormones (Batterham et al., 2003) and limited suppression of ghrelin with feeding (English et al., 2002; le Roux et al., 2005). Therefore, although we found no adaptation of acute appetite regulatory responses in lean individuals following different morning feeding interventions, it is relevant to investigate if the relative stability of acute appetite regulation is present in obese individuals undertaking the same interventions.

The present study aims to investigate whether chronic (6-week) exposure to either a morning fasting or daily breakfast regimen can cause adaptation of acute appetite regulation and metabolic responses throughout the day and energy intake at an *ad libitum* lunch in obese individuals. Based upon previous interventions and the results from our work in lean individuals, we hypothesise that there will be no adaptation of acute appetite regulation and metabolic responses to feeding by either daily morning fasting or breakfast consumption.

8.2 Participants and Methods

8.2.1 Participants

Twenty two, obese men (n = 8) and women (n = 14) aged 25-58 y took part in this study. Participants were recruited via local advertisement from South West England and were initially assessed for eligibility based upon a body mass index of ≥ 30 $kg \cdot m^{-2}$ and then later classified as lean based upon DEXA-derived fat mass indices of ≥9 $kg \cdot m^{-2}$ (men) and ≥13 $kg \cdot m^{-2}$ (women) (Kelly et al., 2009). The study was part of a larger randomised controlled trial entitled the Bath Breakfast Project. In accordance with eligibility criteria set out in Chapter 2, participants reported adhering to a standard sleep-wake cycle (e.g no shift workers) and not anticipating any change in lifestyle during the study period. Participants were free of metabolic disorders, with pre-menopausal female participants either menstruating regularly, or following their chosen contraceptive method (i.e pill, implant) for >6 months. In this study participants were required to report being weight stable (±2 % body mass fluctuation within past 6 months). Within the study cohort there was a mix of regular breakfast consumers (classified as >50 kcal intake within 2 hours of waking on ≥4 days of the week) and non-consumers. Characteristics of participants are presented in Table 8.1.

Written informed consent was obtained from all participants, with the Ethical Approval for the study obtained from the NHS Bristol Research Ethics Committee. The study is registered with Current Controlled Trials [ISRCTN31521726].

Table 8.1: Participant characteristics

Characteristic		Breakfast Group	Fasting Group
n		11	11
Age (y)		43 (10)	44 (10)
Body Mass (kg)		104.0 (24.0)	92.4 (11.2)
Body Mass Index (kg/m^2)		35.0 (6.1)	32.0 (2.3)
Fat Mass Index (kg/m^2)*	**All**	14.8 (5.0)	12.0 (2.3)
	Female	16.9 (4.5)	13.2 (1.8)
	Male	9.9 (1.4)	9.8 (0.8)
Habitual Breakfast Consumers (*n*)		7	6
Female (*n*)		7	7

*Fat mass index calculated as DEXA derived total fat mass divided by height squared. Values represent mean with (SD)

8.2.2 Study Methodology

The study design, experimental protocols and approach to statistical analysis for this investigation were identical to those outlined in Chapter 5 of this thesis. The only difference in this study was the quantity of breakfast provided. The breakfast was again provided in quantities that contained 0.06 g carbohydrate per kcal of each individual participant's measured daily resting metabolic rate and due to the greater body mass (and therefore correspondingly greater RMR) of the obese participants in this study, this resulted in an energy intake of 525 ± 106 kcal and 537 ± 80 kcal in the breakfast and fasting groups, respectively.

8.3 Results

8.3.1 Mass and RMR

Body mass was relatively stable between the two trials, with both groups post-intervention body mass on their second breakfast feeding trial within 0.9 kg of their pre-intervention body mass. RMR was 1613 ± 257 kcal·d^{-1} in the fasting group and 1679 ± 335 kcal·d^{-1} in the breakfast group prior to the intervention. In response to the interventions RMR was stable within 10 kcal·d^{-1}.

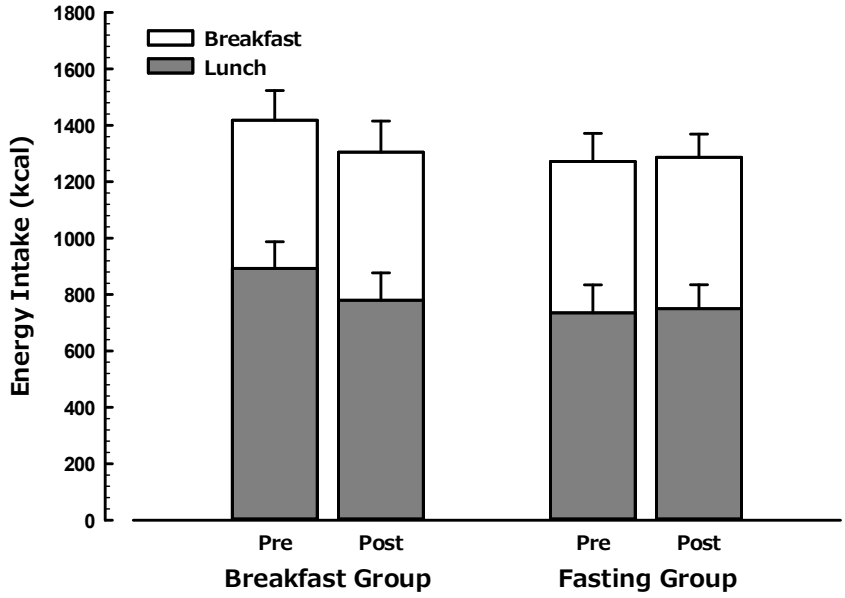

Figure 8.3: Energy intake during the feeding trial before and after 6-weeks of daily breakfast or fasting. Breakfast intake was prescribed, with the breakfast provided for both groups based on RMR similar (Breakfast Group, 525 ± 106 kcal *vs* Fasting Group, 537 ± 80 kcal; $p = 0.78$) and did not change from pre to post-intervention. The error bars on the grey portion of the stack represent the SEM of the energy intake at lunch, and the error bars on the white portion the SEM of the energy intake of the whole day. *Equivalent figure for lean individuals on page 157*

8.3.2 Energy Intake

Energy intake at lunch was not different between groups or trials (both $p > 0.1$), however, there was a tendency for a group x trial interaction in lunch intake ($F = 3.92$, $p = 0.06$). In the breakfast intervention group, lunch intake was reduced by 113 kcal (893 ± 313 *vs* 779 ± 323; $p = 0.05$) after the intervention. Lunch intake was stable (735 ± 330 kcal vs 750 ± 284 kcal) before and after the intervention in the fasting group. Overall intake in those consuming breakfast for 6 weeks was 1418 ± 350 kcal prior to the intervention and 1305 ± 366 kcal following the intervention. In those who underwent the fasting intervention, intake was 1272 ± 331 kcal pre-intervention and 1286 ± 274 kcal post-intervention.

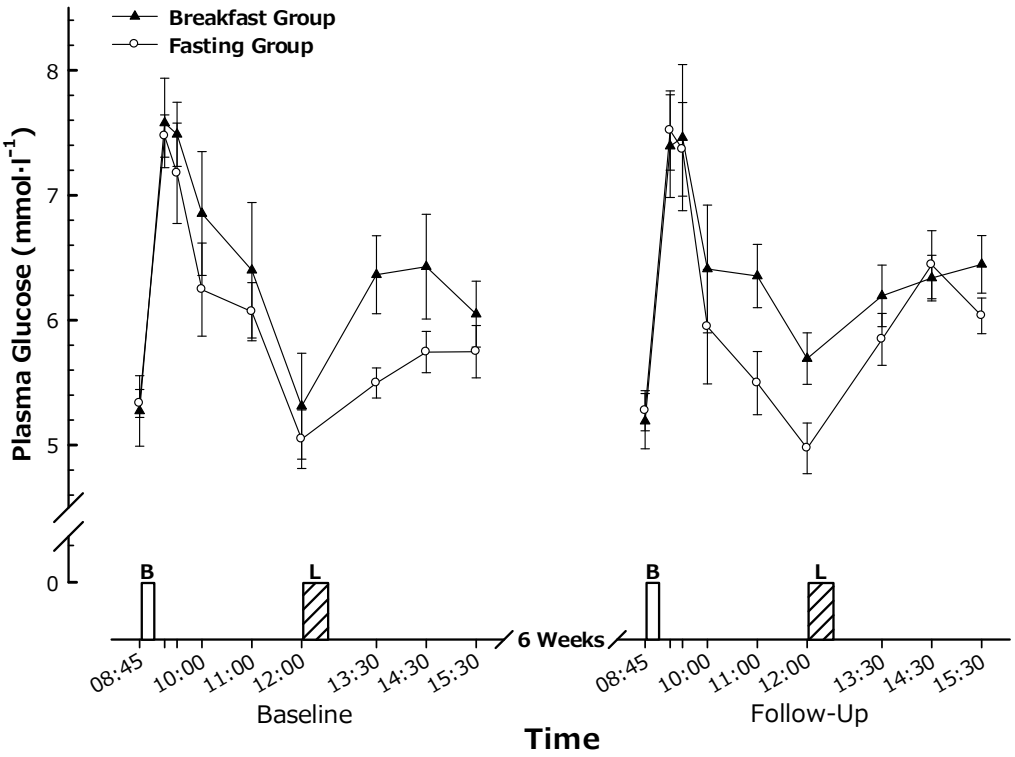

Figure 8.4: Glucose responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L = *Ad libitum* lunch. *Equivalent figure for lean individuals on page 158*

8.3.3 Glucose

For plasma glucose concentrations during the trials there was a main effect of time ($F = 20.2$, $p < 0.01$; Figure 8.4). There were no other main effects or interactions (all $p > 0.2$). Individual peak glucose concentrations were not different between groups or trials (Breakfast Group, 7.93 ± 0.72 mmol·l^{-1} *vs* 7.87 ± 1.29 mmol·l^{-1}; Fasting Group, 7.81 ± 0.72 mmol·l^{-1} *vs* 7.63 ± 1.02 mmol·l^{-1}) and there was no interaction effect (all $p > 0.6$). Similarly there were no main effects or interactions for individual nadir glucose (all $p > 0.6$), which remained stable in response to the intervention (Breakfast Group, 4.90 ± 1.10 mmol·l^{-1} *vs* 4.96 ± 0.89 mmol·l^{-1}; Fasting Group, 4.82 ± 0.57 mmol·l^{-1} *vs* 4.77 ± 0.69 mmol·l^{-1}). Fasting glucose was not different between trials or groups and there was no interaction effect (all $p > 0.4$).

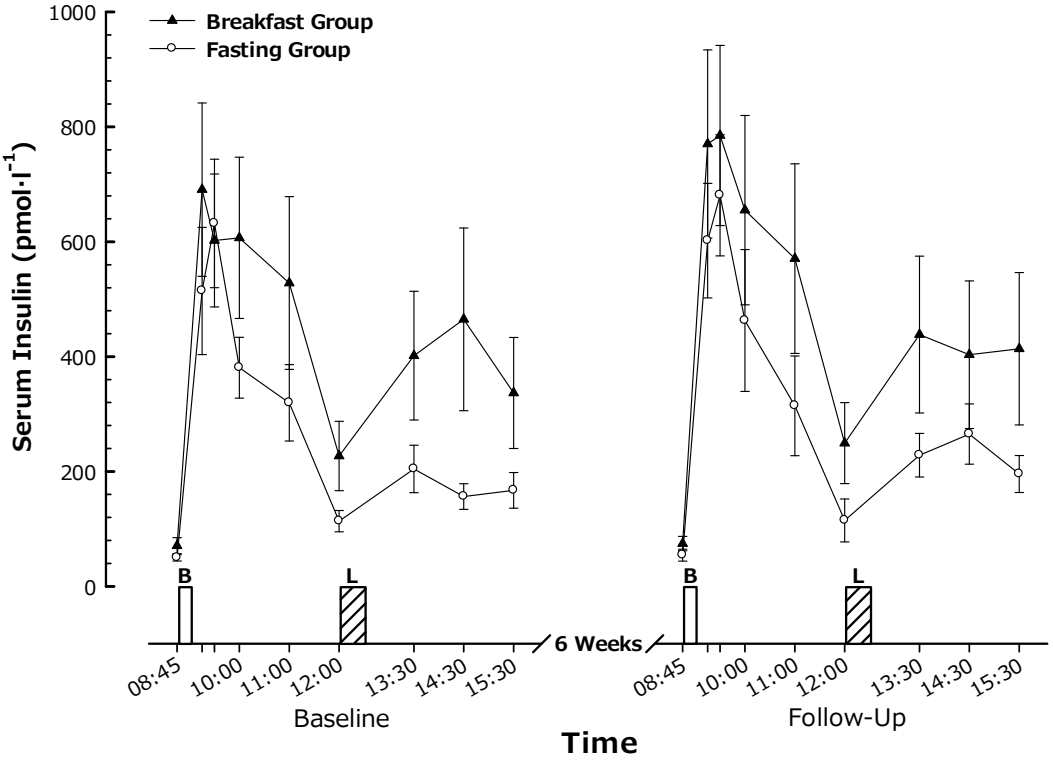

Figure 8.5: Insulin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L = *Ad libitum* lunch. *Equivalent figure for lean individuals on page 159*

8.3.4 Insulin

Serum insulin concentrations varied over time ($F = 19.2$, $p < 0.01$; Figure 8.5) but there were no other main effects or any other interactions (all $p > 0.1$). Individual peak insulin concentrations were not different between groups or trials (Breakfast Group, 813 ± 436 pmol·l^{-1} *vs* 930 ± 470 pmol·l^{-1}; Fasting Group, 730 ± 393 pmol·l^{-1} *vs* 803 ± 426 pmol·l^{-1}) and there was no interaction effect (all $p > 0.1$). Fasting insulin was not different between trials or groups and there was no interaction effect (all $p > 0.1$).

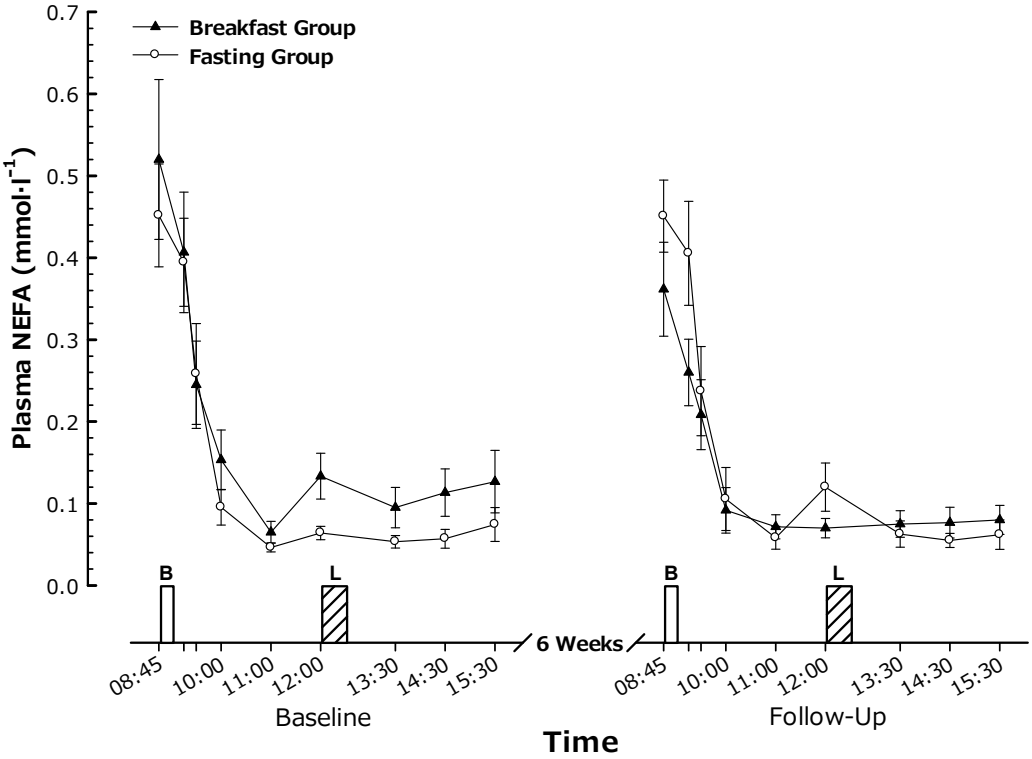

Figure 8.6: NEFA responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L = *Ad libitum* lunch. *Equivalent figure for lean individuals on page 160*

8.3.5 NEFA

A main effect of time was apparent for plasma NEFA concentrations ($F = 52.0$, $p < 0.01$; Figure 8.6) but no other main effects or interactions were evident ($p > 0.2$) apart from a tendency for a trial x group interaction ($F = 3.2$, $p = 0.09$). Peak NEFA concentrations amongst individuals were not different between groups or trials (Breakfast Group, 0.55 ± 0.27 mmol·l^{-1} *vs* 0.43 ± 0.18 mmol·l^{-1}; Fasting Group, 0.47 ± 0.21 mmol·l^{-1} *vs* 0.49 ± 0.20 mmol·l^{-1}) with no evidence of an interaction effect (all $p > 0.1$). Fasting NEFA concentrations were not different between groups or trials and there was no interaction effect (all $p > 0.1$).

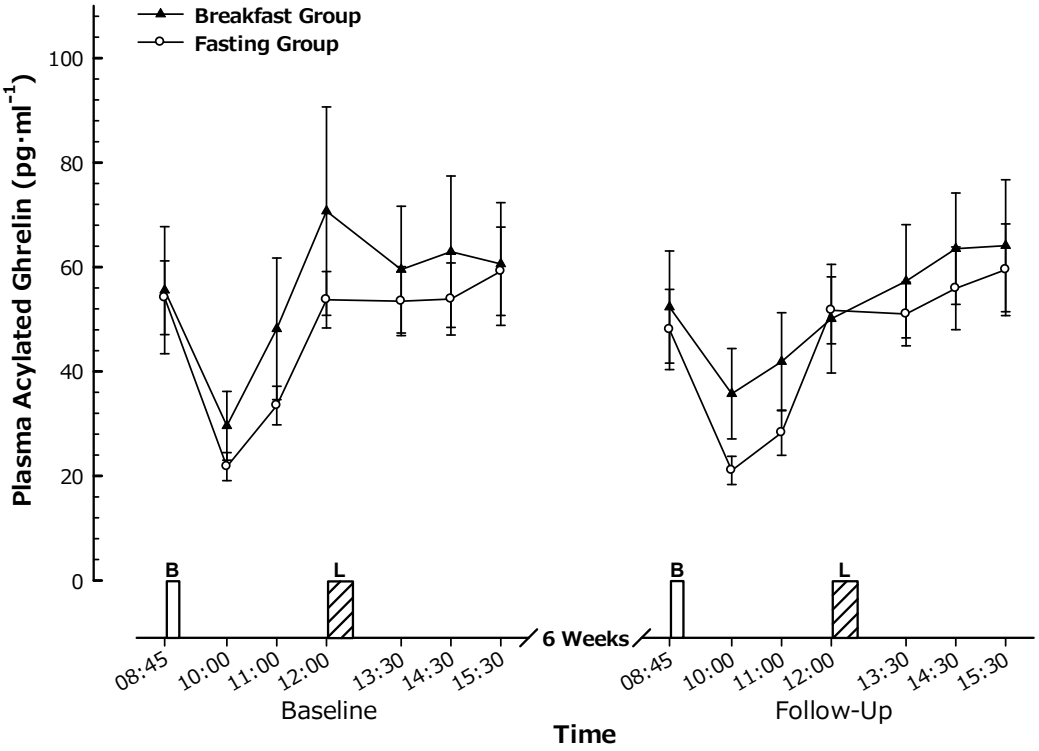

Figure 8.7: Acylated ghrelin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L = *Ad Libitum* lunch. *Equivalent figure for lean individuals on page 161*

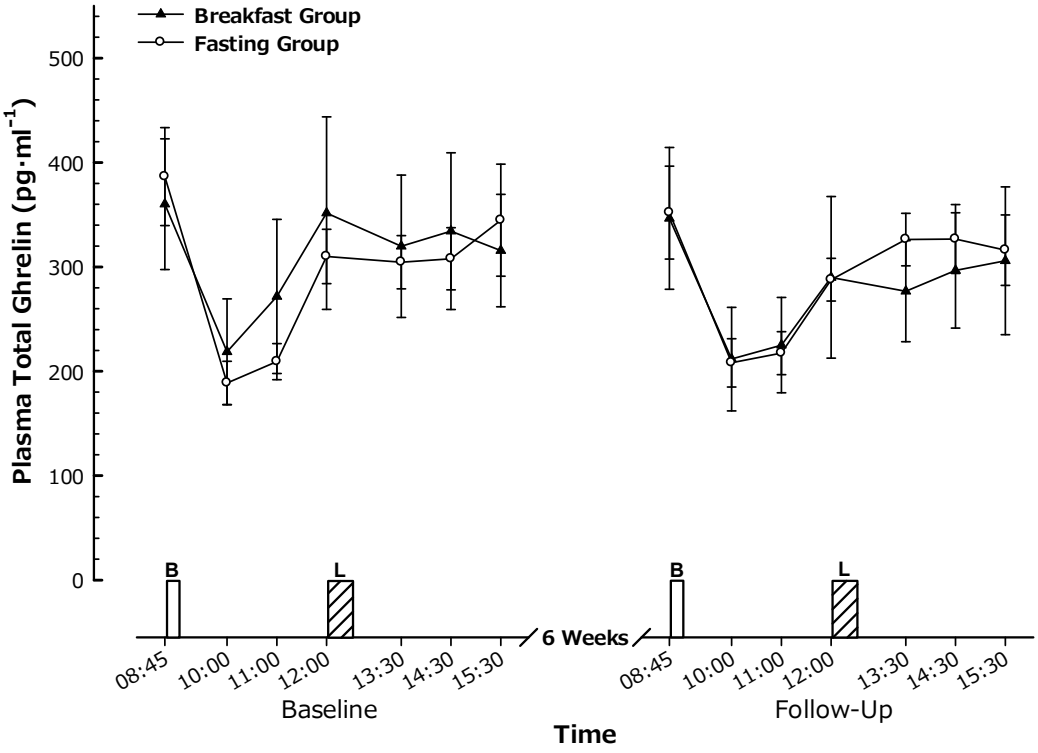

Figure 8.8: Total ghrelin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L = *Ad Libitum* lunch. *Equivalent figure for lean individuals on page 161*

8.3.6 Acylated and Total Ghrelin

Plasma acylated ghrelin concentrations varied over time (F = 27.6, $p < 0.01$; Figure 8.7) but were not different between trials or groups (both $p > 0.3$). There were no interactions (all $p > 0.2$) apart from a tendency for a trial x time interaction (F = 3.0, $p = 0.07$). Individual peak acylated ghrelin concentrations were not significantly different between groups or trials (Breakfast Group, 80 ± 56 pg·ml^{-1} *vs* 68 ± 34 pg·ml^{-1}; Fasting Group, 65 ± 23 pg·ml^{-1} *vs* 65 ± 25 pg·ml^{-1}) with no interaction effect (all $p > 0.3$). Nadir concentrations were similar, with no difference between groups or trials (Breakfast Group, 30 ± 19 pg·ml^{-1} *vs* 35 ± 25 pg·ml^{-1}; Fasting Group, 28 ± 19 pg·ml^{-1} *vs* 33 ± 29 pg·ml^{-1}) with no evidence of an interaction effect (all $p > 0.1$). Fasting acylated ghrelin concentrations were not different between groups or trials and there was no interaction effect (all $p > 0.3$).

Plasma total ghrelin concentrations displayed a main effect of time (F = 17.7, $p < 0.01$; Figure 8.8) but there were no other main effects or interactions (all $p > 0.2$). Individual peaks of total ghrelin concentrations were not significantly different between groups or trials (Breakfast Group, 407 ± 227 pg·ml^{-1} *vs* 355 ± 187 pg·ml^{-1}; Fasting Group, 413 ± 134 pg·ml^{-1} *vs* 416 ± 110 pg·ml^{-1}) with no interaction effect (all $p > 0.2$). Nadir concentrations were similar, with no difference between groups or trials (Breakfast Group, 213 ± 136 pg·ml^{-1} *vs* 201 ± 119 pg·ml^{-1}; Fasting Group, 205 ± 97 pg·ml^{-1} *vs* 226 ± 129 pg·ml^{-1}) with no evidence of an interaction effect (all $p > 0.2$). Fasting concentrations of total ghrelin were similar between groups and trials and there was no evidence of an interaction effect (all $p > 0.2$).

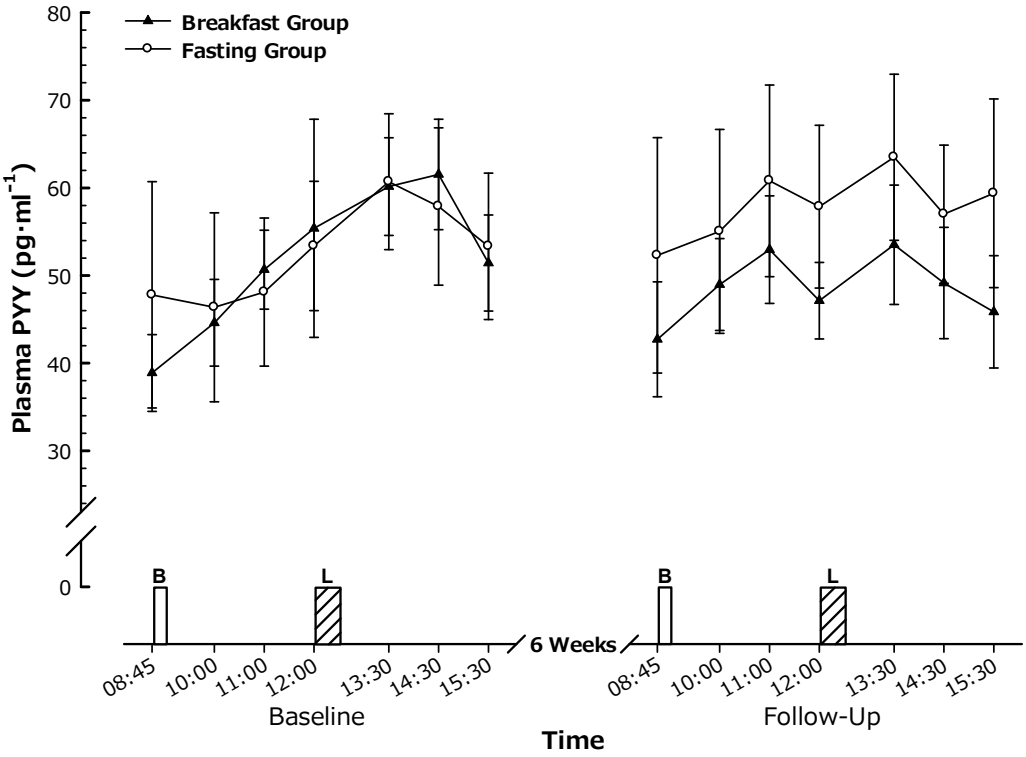

Figure 8.9: PYY responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch. *Equivalent figure for lean individuals on page 163*

8.3.7 PYY

Plasma PYY concentrations were not different over time ($F = 1.89$, $p = 0.17$; Figure 8.9) or between trials or groups (both $p > 0.5$). No interactions were apparent (all $p > 0.1$) apart from a tendency for a trial x group interaction ($F = 4.29$, $p = 0.06$). Individual peaks of PYY concentrations were not significantly different between groups or trials (Breakfast Group, 75 ± 28 pg·ml^{-1} *vs* 65 ± 17 pg·ml^{-1}; Fasting Group, 72 ± 33 pg·ml^{-1} *vs* 78 ± 38 pg·ml^{-1}) with no interaction effect (all $p > 0.1$). Nadir concentrations were similar, with no difference between groups or trials (Breakfast Group, 34 ± 9 pg·ml^{-1} *vs* 34 ± 10 pg·ml^{-1}; Fasting Group, 36 ± 16 pg·ml^{-1} *vs* 40 ± 15 pg·ml^{-1}) with no evidence of an interaction effect (all $p > 0.2$). Fasting PYY was not different between group or trials with no interaction effect (all $p > 0.2$).

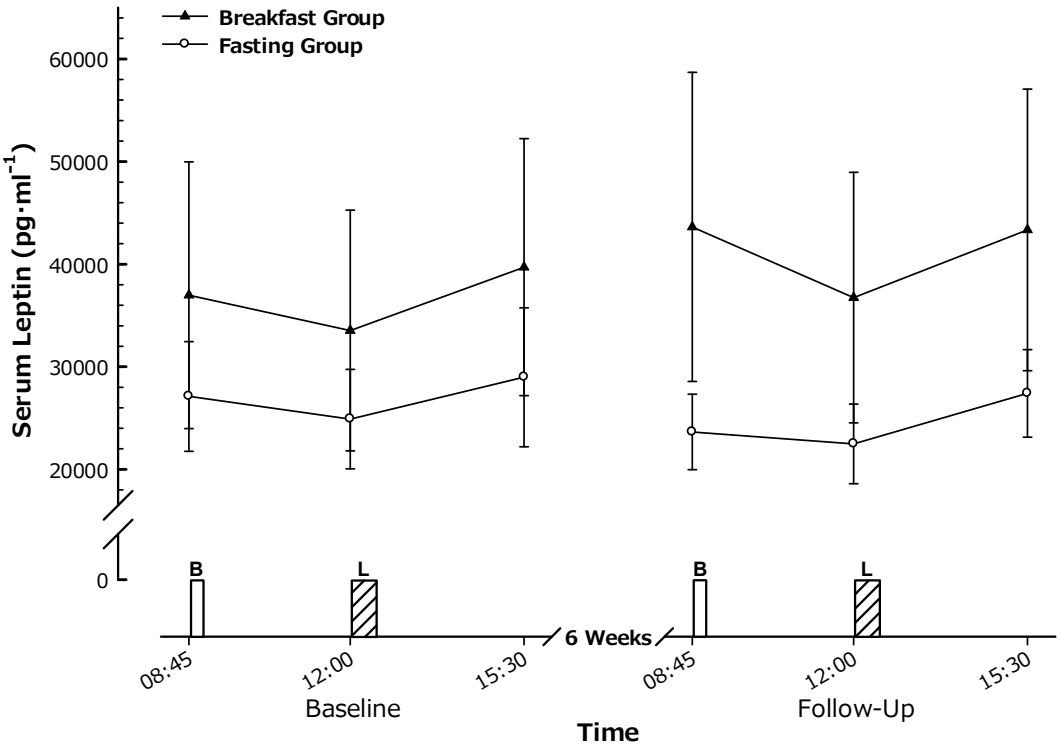

Figure 8.10: Leptin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad Libitum* lunch. *Equivalent figure for lean individuals on page 165*

8.3.8 Leptin

Serum leptin concentrations are displayed in Figure 8.10. There was a main effect of time ($F = 17.39$, $p < 0.01$) but no other main effects or interactions (all $p > 0.1$) for leptin concentrations.

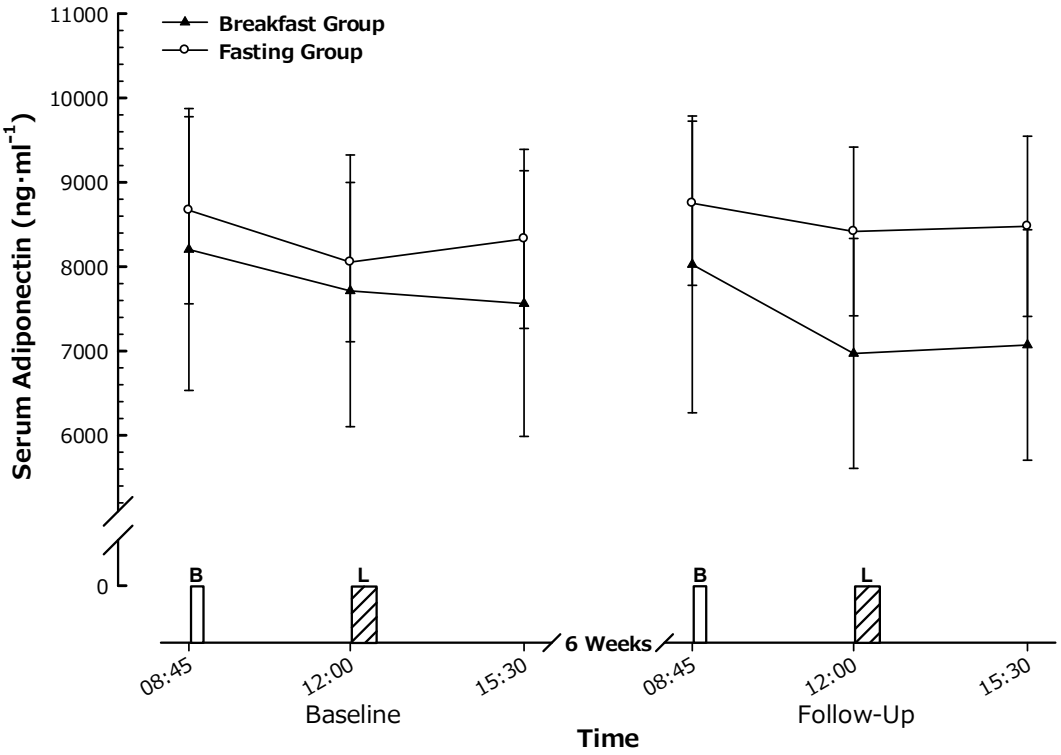

Figure 8.11: Adiponectin responses to feeding before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch. *Equivalent figure for lean individuals on page 166*

8.3.9 Adiponectin

Serum adiponectin concentrations varied over time ($F = 12.91$, $p < 0.01$; Figure 8.11) but there were no other main effects or interactions apparent (all $p > 0.1$).

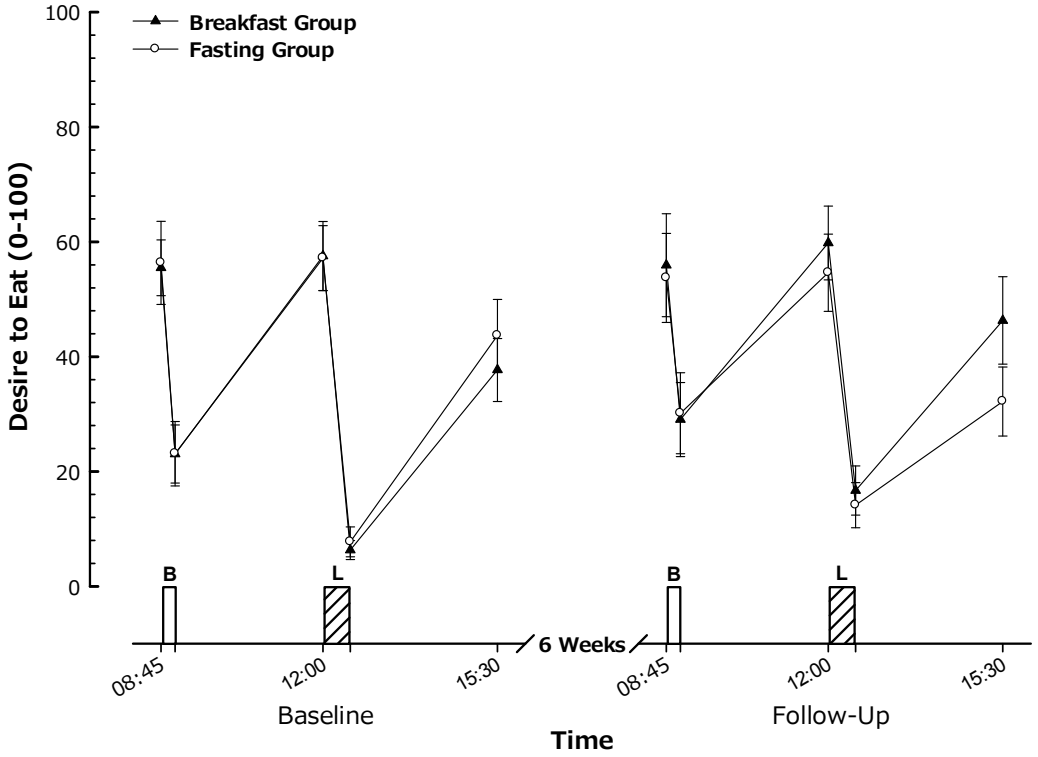

Figure 8.12: Desire to eat during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

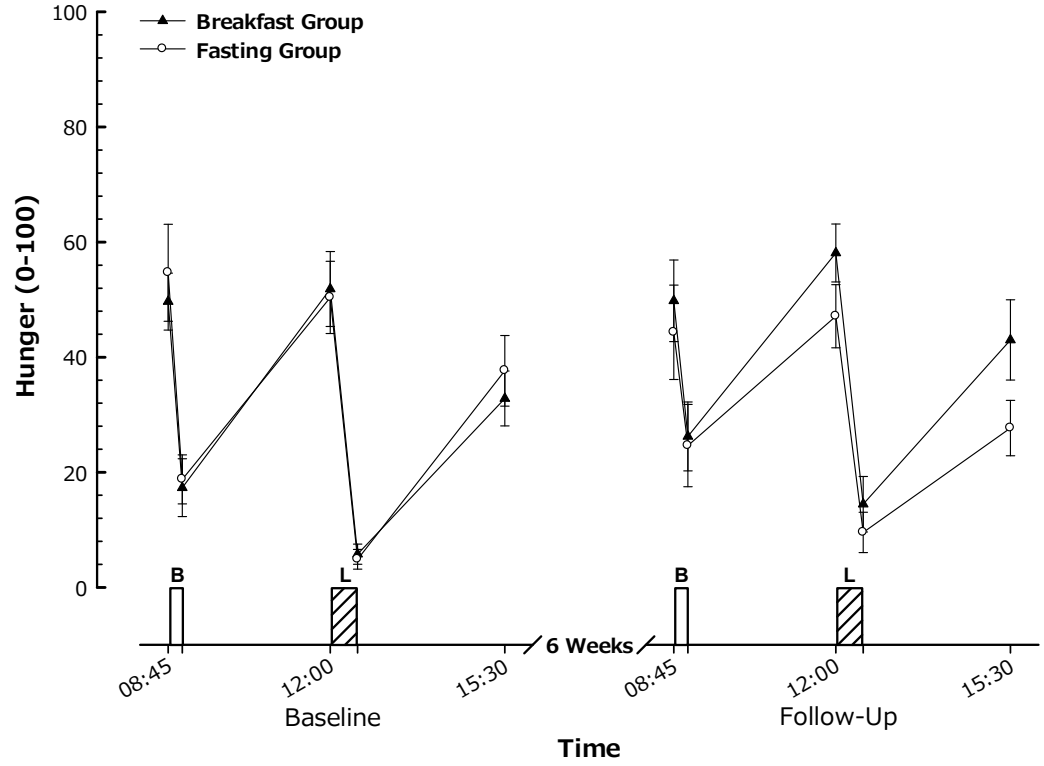

Figure 8.13: Hunger during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

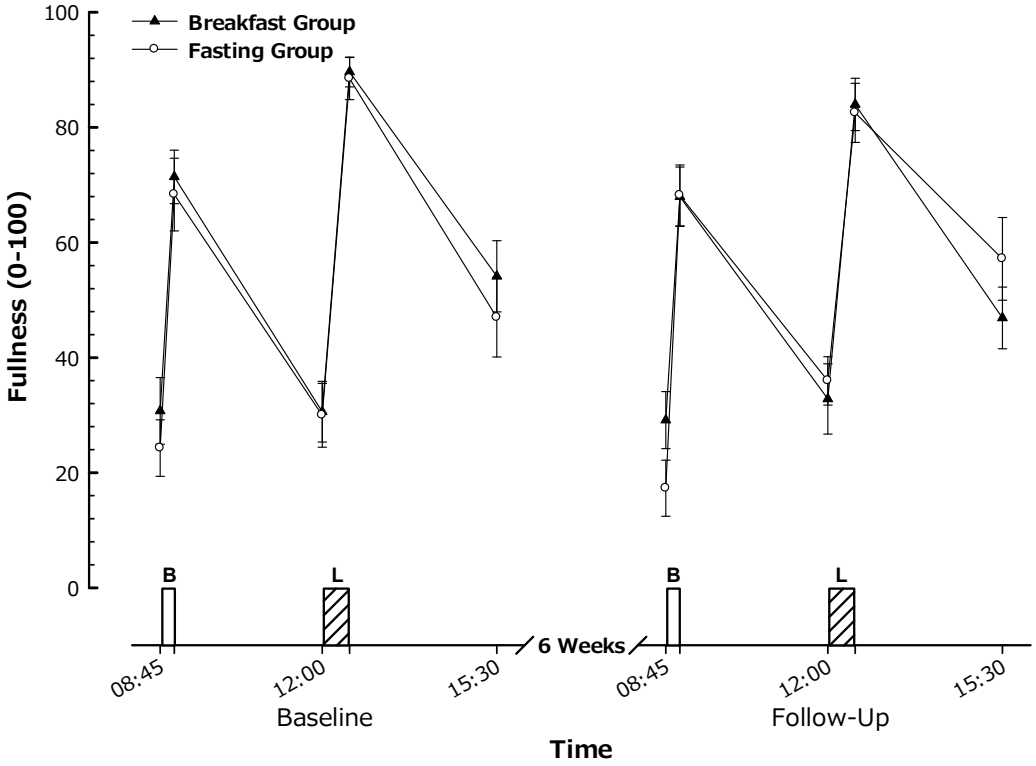

Figure 8.14: Fullness during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

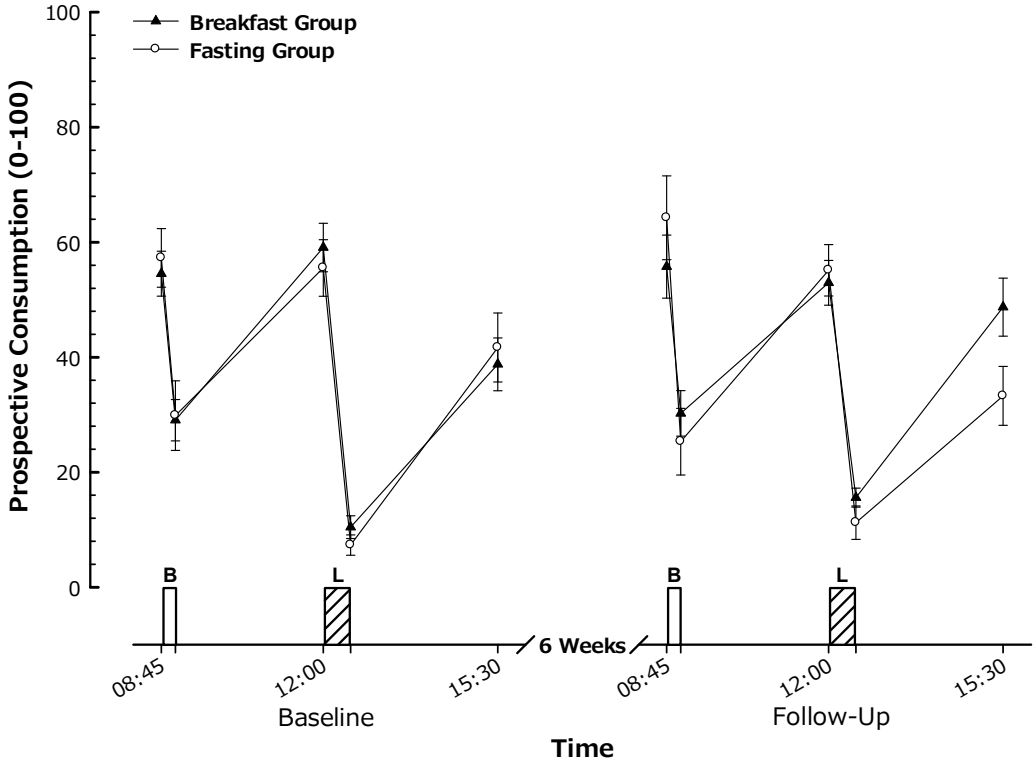

Figure 8.15: Prospective consumption during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch

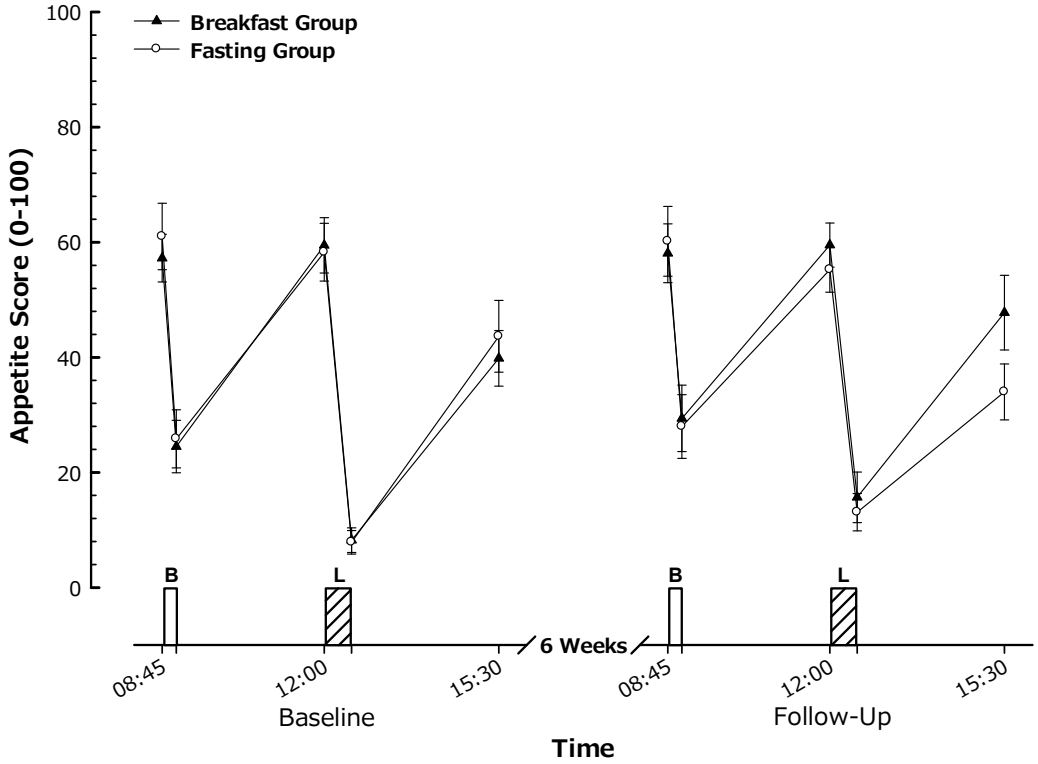

Figure 8.16: Appetite Score during feeding trials before and after a 6 week intervention in the breakfast and fasting groups. B= Prescribed Breakfast, L=*Ad libitum* lunch. *Equivalent figures for appetite ratings for lean individuals on pages 167-169*

8.3.10 Appetite Sensations

Subjective appetite sensations are displayed in Figures 8.12-16. Desire to eat varied over time ($F = 43.5$, $p < 0.01$; Figure 8.12) but there were no other main effects or interactions apparent (all $p > 0.2$). Fasting desire to eat was not different between groups or trials and there was no trial x group interaction (all $p > 0.7$).

Hunger varied over the course of the day ($F = 42.5$, $p < 0.01$; Figure 8.13) but there was no difference between trials or groups (both $p > 0.3$). A trial x group interaction was apparent ($F = 4.39$, $p = 0.05$), with greater hunger in the breakfast consumption group and lesser hunger in the fasting group across the day following the intervention. There were no other interactions for hunger sensations (all $p > 0.2$). Fasting hunger was not different between groups or trials and there was no trial x group interaction (all $p > 0.3$).

Fullness varied over the course of the day ($F = 77.7$, $p < 0.01$; Figure 8.14) but was not different between trials or groups and no interactions were evident (all $p > 0.2$). Fasting fullness was not different between groups or trials and there was no trial x group interaction (all $p > 0.1$).

There was a main effect of time for prospective consumption ($F = 49.0$, $p < 0.01$; Figure 8.15) but no other main effects or any interactions (all $p > 0.1$). Fasting prospective consumption was not different between groups or trials and there was no trial x group interaction (all $p > 0.3$).

The combined appetite score varied over time ($F = 57.4$, $p < 0.01$, Figure 8.16). There were no other main effects of trial, group or any other interactions (all $p > 0.l$). The fasting appetite score was not different between groups or trials and there was no trial x group interaction (all $p > 0.5$).

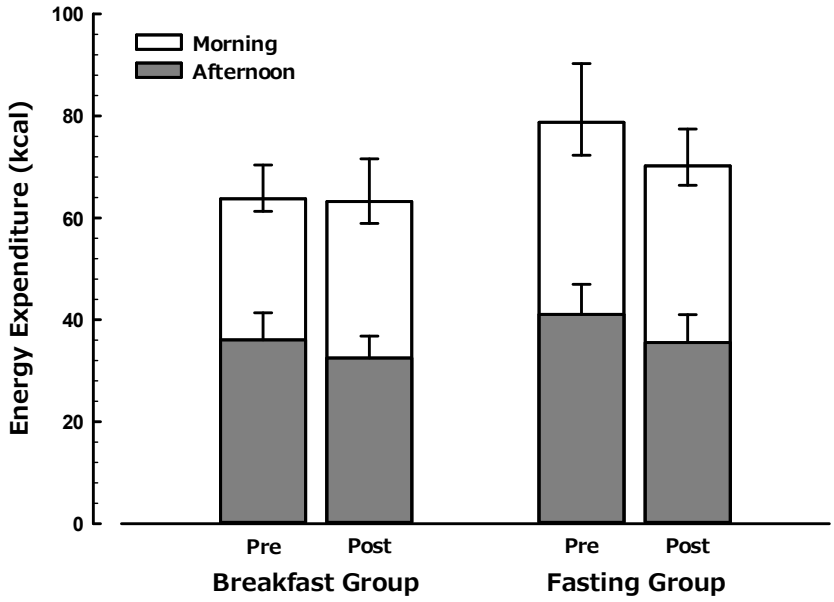

Figure 8.17: Diet induced thermogenesis before and after 6-weeks of daily breakfast or fasting. Error bars reflect SEM. Asymmetric error bars are plotted on the morning section of each stack. The negative portion of these error bars reflects the SEM of the morning period and the positive portion the SEM of the whole day. *Equivalent figure for lean individuals on page 171*

8.3.11 Diet Induced Thermogenesis

Diet induced thermogenesis in the morning did not differ between trials or groups with no interaction effect (all $p > 0.2$). For the afternoon period there was no difference in DIT between trials, groups or a trial x group interaction (all $p > 0.1$). When these periods were combined to establish DIT for the whole day, there was no difference between trials, groups or an interaction (all $p > 0.3$).

8.4 Discussion

The present study aimed to investigate the impact of a 6 week intervention manipulating morning feeding patterns upon acute energy intake, appetite regulation and metabolic responses to feeding in a laboratory based protocol in obese individuals. Following a standardised breakfast there was a tendency for those who had undertaken a daily breakfast regimen to consume less at an *ad libitum* lunch following their intervention. There was no indication of altered postprandial insulin sensitivity in either group and the majority of appetite hormone responses remained stable following the intervention, apart from a tendency for divergent PYY responses between groups. Diet induced thermogenesis was not affected by either intervention. Subjective appetite responses indicated greater perceptions of hunger following the breakfast consumption intervention with reductions following daily fasting; however, it is unclear whether this response is a direct product of reduced energy intake at the *ad libitum* lunch in the breakfast group or meaningful adaptation to chronic exposure to the intervention. This work does not provide any evidence for negative metabolic consequences to feeding or increased hunger or acute energy intake in individuals that followed a daily morning fasting regimen for 6 weeks.

In contrast with our lean participants there was some evidence of reduced energy intake during the *ad libitum* lunch following adherence to the daily breakfast consumption regimen. The reduction observed is somewhat surprising considering that there was greater reported subjective hunger prior to lunch following the daily breakfast regimen. Therefore, either the food provided at lunch became more satiating following the intervention (explaining the reduced intake) or this provides more evidence of a potential dissociation between subjective measures of appetite and objective measures of intake in obese individuals (Flint et al., 2007). In a free-living setting described in Chapter 7, there was reduced reported dietary intake between weeks 1 and 6 to both the breakfast consumption and fasting interventions in the same cohort as the present study. This potentially indicates that either the food diaries may have lacked sensitivity to detect differences between the conditions, or that whilst energy intake at lunch may have been different (not an analysis that has been conducted) that any differences apparent may have been compensated for, either with differing food choices/intakes at other meals or increased snacking.

Similar to our findings in lean individuals in Chapter 3, appetite hormone responses throughout the day were not affected by either the daily breakfast or morning fasting regimen. This appears to highlight that despite several investigations identifying different concentrations/patterns of response of appetite hormones in obese individuals (Batterham et al., 2003; Carroll et al., 2007; English et al., 2002; le Roux et al., 2006; le Roux et al., 2005; Shiiya et al., 2002; Tschop et al., 2001b), these regulatory hormones do not exhibit any adaptation to differing daily morning feeding regimens. This reinforces that feeding pattern seems to have little impact upon appetite hormone responses to feeding in the context of relative weight stability.

In those individuals who undertook the daily breakfast intervention, hunger was greater at the end of the testing day following the intervention. However, as discussed, participants also consumed less at the *ad libitum* lunch. Therefore, it is unclear whether this is a product of the reduced intake or an effect of the breakfast intervention in exacerbating the return of hunger after eating (of which there is some tentative evidence, as hunger was also increased 3 hours after the fixed breakfast meal in the breakfast group after the intervention). Whilst our current design provides valuable information with regards to self-selected intake, to establish the specific effects on subjective appetite of feeding interventions, fixed meals would be appropriate to remove this potential covariate and allow direct comparisons of the satiating effect of foods before and after long term interventions.

A previous investigation in lean women has resulted in reduced insulin sensitivity in response to a 2 week intervention where the first feeding of the day was delayed until 11am (Farshchi et al., 2005b). Our current investigation does not replicate these findings, as insulin concentrations were unaffected by either intervention. However, it has previously been contested that changes in fasting insulin sensitivity to feeding frequency interventions may be less apparent in obese individuals as they are likely to already have some degree of insulin resistance (Farshchi et al., 2005a).

This explanation for a lack of effect in those that undertook the fasting intervention is unlikely in this instance, as changes have not been observed in lean individuals undertaking a similar intervention as described in Chapter 5. One methodological difference between the studies is the use of arterialised blood sampling

(Gallen and Macdonald, 1990) in the work of Farshchi and colleagues. While this would be expected to yield greater concentrations of glucose than venous sampling (McGuire et al., 1976; Nauck et al., 1992; Kurpad et al., 1994), there is no evidence to suggest that the pattern of response (or the response to an intervention in a repeated measures design such as the current work) should be different between the two techniques. Therefore it is unlikely that the discrepant results between these studies are attributable to differences in blood sampling methodology.

The same research group have also reported reduced insulin sensitivity (Farshchi et al., 2004b) and dietary thermogenesis in both lean (Farshchi et al., 2004a) and obese (Farshchi et al., 2005a) women following irregular meal patterns. In the latter studies, the authors have attributed the reduced DIT as linked to the reduced insulin sensitivity induced by their interventions, due to the established links between these two phenomena (Ravussin et al., 1985a; Ravussin et al., 1983; Ravussin et al., 1985b). It is therefore as would be expected that in the current investigation there was no change in DIT following either the breakfast or fasting regimen, as insulin concentrations in response to the meals fed were unaffected in both groups.

This study has found that no adaptation of appetite regulatory hormone or metabolic responses to a fixed breakfast or *ad libitum* lunch, following 6 weeks of daily breakfast or morning fasting. There was a trend towards reduced lunch energy intake but greater hunger following the breakfast regimen. The morning fasting intervention did not result in reduced postprandial insulin sensitivity or diet induced thermogenesis. In conclusion, this work does not provide any evidence that a morning fasting intervention negatively affects metabolic responses to feeding but adherence to daily breakfast consumption may, after prolonged exposure, facilitate reduced energy intake at lunch despite increased hunger in obese adults.

Chapter 9: General Discussion

9.1 Overview

The overall aim of this thesis was to examine the effect of extended morning fasting upon energy balance and human health. In order to do this, a mixture of laboratory-based and free-living intervention studies were conducted in both lean and obese humans.

Specifically, Chapter 3 investigated the acute appetite, metabolic and hormonal responses to extended morning fasting and breakfast consumption using a laboratory based feeding protocol in lean participants. Chapter 4 then examined the impact of a 6-week free-living morning fasting or daily breakfast intervention upon all components of energy balance and selected metabolic outcomes and health markers in the same participants. Chapter 5 then established the effects of the two interventions described in Chapter 4 upon the acute responses to breakfast consumption first described in Chapter 3. Chapters 6,7 and 8 then repeated this progression of studies but in an obese cohort.

9.2 Summary of Findings

Lean Individuals

Chapter 3

- Overall energy intake was reduced when extending the morning fast, despite greater consumption of an *ad libitum* lunch.
- The suppression of ghrelin after lunch consumption was blunted following breakfast consumption, but PYY and leptin concentrations were greater.
- Insulin concentrations were substantially greater in response to lunch intake following morning fasting.
- There were no differences in subjective appetite ratings during the afternoon.

Chapter 4

- Daily total physical activity energy expenditure was lesser in those asked to extend their overnight fast, an effect most consistent in the morning.
- Energy intake in those fasting during the morning was substantially lower than those eating breakfast, indicating little compensation for prescribed intake throughout the rest of the day in those consuming breakfast.
- Metabolic outcomes and health markers were mostly unaffected by either intervention.

Chapter 5

- Energy intake at an *ad libitum* lunch meal was not significantly affected by adherence to either an extended morning fasting or daily breakfast regime.
- Metabolic and appetite hormone responses throughout the day were generally unaffected by either chronic intervention.
- Subjective appetite responses were unaffected.

Obese Individuals

Chapter 6

- Overall energy intake was reduced when extending the morning fast, with no difference in intake at an *ad libitum* lunch.
- The suppression of ghrelin after lunch consumption was blunted following breakfast consumption, but PYY and leptin concentrations were greater.
- Insulin concentrations were substantially greater in response to lunch intake following morning fasting.
- There were no differences in subjective appetite ratings during the afternoon.

Chapter 7

- Daily total physical activity energy expenditure was similar in both intervention groups, although physical activity energy expenditure was greater prior to 12:00 in those consuming breakfast.
- Energy intake in those fasting during the morning was similar to those eating breakfast, indicating additional intake throughout the rest of the day.
- Metabolic outcomes and health markers were mostly unaffected by either intervention.

Chapter 8

- Energy intake at an *ad libitum* lunch meal was reduced by adherence to a daily breakfast regime.
- Metabolic and appetite hormone responses throughout the day were generally unaffected by either chronic intervention.
- Subjective appetite responses were mainly unaffected but there was some indication of increased hunger perceptions after the daily breakfast regime.

9.3 Effect of Morning Fasting upon Energy Balance

Ultimately, the components of energy balance discussed throughout this thesis all contribute to body mass change/stability over the long term. Below is the effect of the 6 week morning fasting and daily breakfast consumption interventions on body mass change in both lean and obese individuals (Figure 9.1). As can be seen, the lean and obese cohorts displayed differing responses to the feeding interventions, with weight loss in both intervention groups in lean individuals and the opposite in obese.

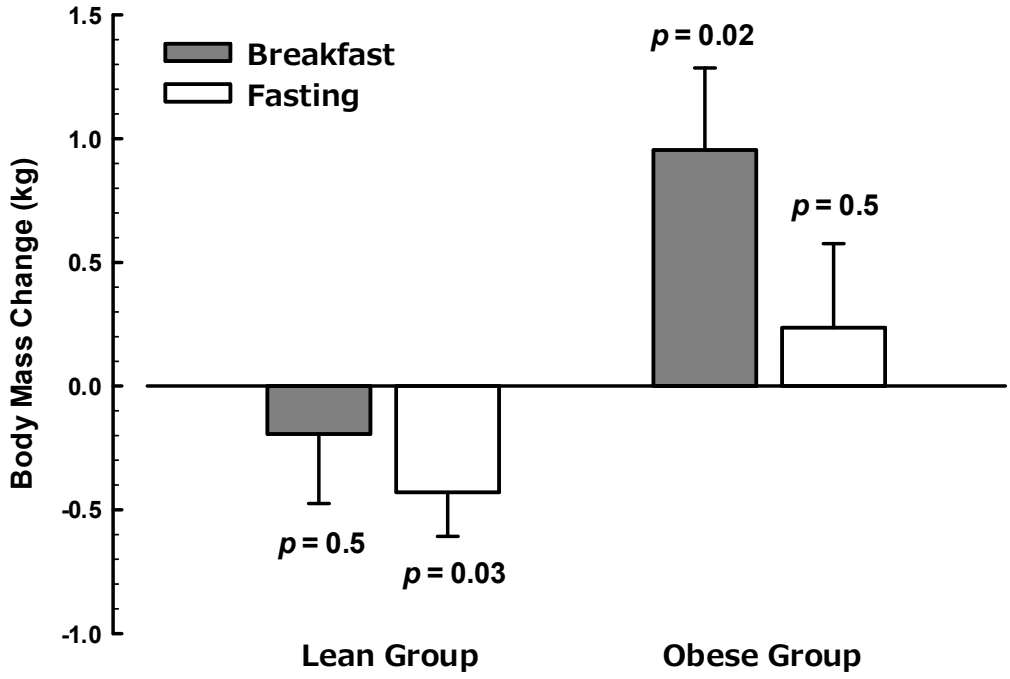

Figure 9.1: Body mass change in both groups during the breakfast and morning fasting interventions. *p* values on the figure represent the pre-post comparisons of body mass change within each group.

While the individual interaction effects for weight were not significant in either lean or obese groups, simple pre-post contrasts within experimental groups identified that lean individuals omitting breakfast lost weight and obese individuals consuming breakfast gained weight. It therefore appears that lean individuals do not fully compensate for "missed" intake and obese individuals do not compensate for "added" intake (i.e the requirement to consume 700 kcal daily by 11:00 in the breakfast consumption group). Whether these changes in weight are considered of a relevant magnitude or of statistical significance are secondary to the simple conclusion that

regardless of weight status, those asked to extend their morning fast either lost more weight (lean) or gained less weight (obese) than counterparts asked to consume a self-selected breakfast daily during the interventions. This is contrary to the strongly engrained popular health message that breakfast consumption is facilitative in managing weight (Brown et al., 2013) and also partially agrees with recent evidence suggesting no effect of breakfast recommendations upon weight change (Dhurandhar et al., 2014a).

9.4 Acute Appetite Regulatory Responses

In this thesis, short-term regulation of appetite and metabolic responses to feeding has been examined in both lean and obese individuals. The most striking finding from Chapters 3 and 6 is the similarity of the acute responses to morning fasting and breakfast in lean and obese individuals. The pattern of appetite regulatory hormones is consistent between the two populations, with the only difference for the majority of hormones being the concentrations of the hormones present. For PYY, this finding is relatively consistent with previous investigations that have reported lower concentrations of the hormone in obese individuals (Batterham et al., 2003; le Roux et al., 2006). However, despite acylated ghrelin concentrations being reduced in our obese cohort as would be expected (Tschop et al., 2001b; Shiiya et al., 2002), the patterns of response to feeding were in contrast with some evidence to suggest differential responses for ghrelin between lean and obese individuals (le Roux et al., 2005; English et al., 2002). The consistency of these responses in lean and obese individuals seems to suggest that dysregulation of appetite hormone secretion to acute feeding/fasting may not be an important factor in the pathogenesis of obesity. Considering the complexity of appetite regulation, it appears that altered homeostatic regulation of energy intake may not be a probable explanation for development of obesity, and other factors (e.g hedonic regulation, societal and environmental influences) might be more prescient.

The work in Chapters 5 and 8 also provide minimal evidence that acute responses to feeding can be altered by either adherence to a morning fasting or daily breakfast intervention in either lean or obese individuals. This work combined with other similar findings manipulating diet/feeding frequency in the literature (Cameron et al., 2010; Carlson et al., 2007; Ellis et al., 2012) seem to suggest that subtle manipulations of diet have little impact upon these hormonal responses to feeding.

9.5 Energy Intake in Laboratory and Free-Living Settings

As has been discussed throughout the thesis, appetite regulation is highly complex. Whilst the laboratory protocols employed in Chapters 3,5,6 and 8 allow us to examine in detail the effect of altered food intake patterns on some mechanisms of appetite regulation; by design, these protocols limit the potential influence of other relevant factors in "real world" regulation of energy intake. This is where the free-living protocols employed in Chapters 4 and 7 can provide insight into how basic physiological mechanisms explored in the laboratory may impact upon energy intake when other contributory factors are not controlled. In this regard, it is interesting to compare the relative agreement (or lack thereof) between the acute laboratory and free-living responses in lean and obese individuals.

In lean participants, Chapters 3 and 4 showed relatively consistent energy intake responses in the laboratory and free-living. In both situations, there was evidence of some compensation for "missed" intake due to extended morning fasting, both in the laboratory fasting trials through increased lunch intake (Chapter 3) and in those individuals assigned the morning fasting intervention through a strong tendency ($p = 0.06$) for greater intake after 12:00 relative to the daily breakfast group (Chapter 4). However, in both of these situations the additional intake in those that extended their fast was not sufficient to compensate for the intake through breakfast (i.e both in the laboratory and during free-living, total energy intake was greater when consuming breakfast). In contrast, obese individuals did not demonstrate significantly increased energy intake at an *ad libitum* lunch in the laboratory setting when asked to extend their morning fast (Chapter 6), but those that undertook the morning fasting intervention had sufficiently greater energy intake after 12:00, such that total daily energy intake was not different between the two intervention groups.

The discrepancy between the two groups suggests that energy intake in obese individuals may be more strongly influenced by environmental factors than lean individuals (Mela, 2006). The energy intake compensation that is evident in the obese cohort may be due to food choices and frequency, as opposed to energy intake increases at single homogenous meals as provided in the acute laboratory setting. Alternatively, it could be that free-living energy intake in obese individuals was underestimated in those that were consuming breakfast. This is a possibility that

cannot be ruled out, but that contention is also the case for each experimental group independent of their weight status (although underreporting of EI is greater in obese individuals) or experimental group allocation. Therefore, it seems unlikely that this issue should disproportionately affect one experimental group more than any others (i.e that obese individuals in the breakfast group should underreport energy intake to a greater extent than those in the obese fasting group) (De Castro, 1994b).

9.6 Energy Expenditure during Free-Living Morning Fasting and Daily Breakfast Interventions

In addition to establishing the acute responses to morning fasting and breakfast consumption, this thesis has examined the impact of free-living interventions in lean and obese individuals. Reported energy intake responses to the two interventions were different in lean and obese individuals. Similarities were evident in the relative stability of RMR to both interventions in both groups of participants, indicating that altering morning feeding frequency in the absence of substantial weight change has no impact upon resting metabolic rate. The other major contributor to energy expenditure is the daily physical activity expenditure measured during the intervention, which was found to be greater in those that consumed breakfast than those extending their fast in lean individuals, but was not different between intervention groups in obese individuals. However, in both lean and obese individuals, energy expenditure prior to 12:00 was greater in those consuming breakfast.

As discussed previously in Chapter 7, this effect is unlikely to simply be a product of the DIT due to breakfast consumption and therefore may reflect in both populations limited physical activity in those with restricted energy intake during that period. This is an area that warrants further investigation, as the relationship between temporary restriction of energy intake and physical activity has not received attention. Future investigations should also employ a baseline measure of physical activity to establish if the differences between those fasting during the morning are a result of increased physical activity in those that consume breakfast daily, or a reduction in physical activity during the morning in those fasting. The second possibility would appear more probable as the majority of individuals consume foods in the morning and therefore any response is more likely to occur in the less accustomed intervention.

9.7 Effect of Normal Breakfast Habits upon Outcomes

The present thesis was not designed to investigate the influence of participants' habitual eating behaviours upon the outcomes examined. Due to the intensive nature of the studies undertaken, the work presented in this thesis used participant numbers sufficient to examine the outcomes measured in the intervention groups as a whole. However, while the randomisation scheme employed controlled for the impact of major imbalance in habitual breakfast consumers and skippers, the numbers of individuals did not allow a meaningful comparison of the effect of these pre-randomisation habits upon response to the interventions.

The relevance of pre-randomisation breakfast consumption habits upon response to interventions was first introduced by Schlundt and colleagues in a seminal 1992 publication that examined the effect of breakfast consumption/omission and a behavioural intervention upon weight loss, in individuals prescribed energy restricted diets. They reported that there was a tendency (p = 0.06) for those swapping their normal habits to lose more weight than those continuing their normal lifestyle. However, it has been subsequently suggested that participants' normal breakfast habit has no impact upon weight change in a less prescriptive extended fasting intervention (Dhurandhar et al., 2014a).

As well as this lack of agreement within the literature, it is also pertinent to consider the actual relevance of pre-randomisation habits. There are a number of potential issues with inferring that habitual breakfast consumption can moderate responses to interventions manipulating morning feeding. Firstly, as introduced in Chapter 1, omission of breakfast is associated with a number of behaviours that are detrimental for health (e.g greater alcohol consumption, lower physical activity etc..). Therefore, as assessment of breakfast consumption habits are cross-sectional in nature it is likely that any moderating effect of breakfast consumption/omission would be partly contributed to by these other factors and not necessarily reflect a true effect of prior habitual breakfast consumption.

Secondly, as also discussed in Chapter 1, the classification of habitual breakfast consumption or skipping is highly variable amongst different studies. This arises from several studies employing either self-report measures of breakfast habits

(e.g simply asking participants without any established criteria whether they consume breakfast or not) or asking individuals the frequency of their consumption. However, beyond these issues of methodological consistency there is a wider point that has to be acknowledged; that is, that definitions of breakfast "consumers" and "skippers" are essentially arbitrary. While they provide convenient ways of categorising individuals for studies it is highly unlikely that any differences in physiology between these groups would be so dichotomous. Future work should look to move beyond categorical definitions of these habits and develop "dose-response" relationships between habitual breakfast consumption size/frequency/type and any associated differences in measured outcomes.

9.8 Assessment of Energy Balance

Amongst both the lean and obese participants studied, measured energy intake was less than energy expenditure in both lean and obese participants, such that the discrepancy did not correspond to the magnitude (in lean) or direction of weight change (in obese) in either group. It is therefore probable that there was some underreporting of energy intake. This is not an uncommon phenomenon in applied nutrition research (Livingstone and Black, 2003), and there have previously been methods suggested for the removal of energy intake records deemed implausible (Goldberg et al., 1991). However, in this investigation as these measures generally rely upon achievement of energy balance in participants, these measures were not employed, as the intervention employed meant that energy balance was not necessarily to be expected. Additionally, because of the laborious nature of recording of energy intake (and therefore to minimise the aforementioned underreporting), these diaries were only kept during the first and final week of the interventions participants undertook. Therefore, the food diaries employed may well have reflected the intake during the weeks of measurement, but energy intake may have changed in the weeks where participants were not being monitored.

A possible solution to minimise the effect of dietary underreporting is to employ doubly labelled water for long term assessment of energy expenditure and obtain repeated measurements of weight to enable assessment of overall energy balance (Hall, 2010). Methods based around estimation of energy intake using these methods are referred to as the intake-balance method (Racette et al., 2012). These methods have been shown to be accurate in estimating energy intake during both weight gain (Gilmore et al., 2014) and weight loss (de Jonge et al., 2007). Despite the potential usefulness of this method for estimating total energy intake, this approach would not allow any assessment of macronutrient intake or feeding frequency. Therefore, ongoing assessments of dietary intake will have to balance the need for precision in total energy intake estimation *versus* the desire for additional information relating to the nature of energy intake. Future investigations might attempt to employ a combination of traditional food diaries and the intake-balance method to potentially scale total energy intake on an individual basis but still garner some insight into reported food intake patterns (with the associated limitations).

9.9 Future Directions

There has been limited use of fMRI to assess neural responses to breakfast skipping and differing breakfast types in adolescents (Leidy et al., 2013; Leidy et al., 2011; Leidy and Racki, 2010). This work is also being extended to examine the specific neural responses to administration of various appetite regulating hormones (Batterham et al., 2006; De Silva et al., 2011; Goldstone et al., 2014; Malik et al., 2008). Future investigations should attempt to investigate the neural responses to both acute breakfast consumption/fasting in adults but also examine the effect of long term extended fasting/breakfast interventions upon acute neural responses to feeding. This will extend the work completed in Chapters 5 and 8, to see if adherence to different morning feeding interventions causes adaptation of neural responses to exposure to food cues or consumption.

Two key problems arise when studying appetite and energy intake that must be reconciled to attempt to gain further insight into appetite regulation. As discussed throughout, laboratory studies tend to control external cues to attempt to study hormonal regulation of energy balance. However, the interaction of external cues and our hormonal/neural regulatory processes combine to ultimately result in feeding behaviours. In attempting to study these behaviours in a free-living environment we lose the ability to measure some aspects of appetite regulation (e.g hormonal responses) and lose precision in measuring the ultimate outcome we are interested in (energy intake). Future laboratory investigations should attempt to create as "natural" an environment as possible to allow the assessment of appetite in externally valid settings. For example, buffet style meals should be employed following extended morning fasting to establish if greater energy compensation occurs through selection of different food items at meals, as well as designs incorporating opportunities for snacking as well as meals.

It is important to acknowledge that the work in this thesis is only a first step in attempting to establish the effects of breakfast consumption in energy balance and health. As discussed in the literature review and throughout, there is a paucity of literature examining the impact of extended fasting and as such this was the condition of most interest in the present work. Therefore, whilst there is little possible variability of the implementation of morning fasting, the potential for a multitude of different

breakfast conditions is apparent. The work in this thesis took a pragmatic approach to the prescription (or relative absence of) the breakfast intervention. Future investigations should examine the relative merits of varying compositions and sizes of breakfast in free-living individuals. Indeed, some emerging work is beginning this process (Adamsson et al., 2014; Rabinovitz et al., 2014), although the focus of these investigations have so far been on disease risk factors, studies examining control of energy balance with varying breakfast compositions warrants further attention.

Future work should also attempt to further differentiate beyond the simple groupings (i.e lean or obese) used in these studies. As discussed in Chapter 1 both gender and dietary restraint may play a role in modifying appetite regulation. Neither of these factors were examined in the current investigation so it is currently unclear whether they may modify the response to either acute or chronic extended morning fasting. In the case of gender, the randomisation scheme accounted for gender to ensure equivalent distribution of males and females in each experimental group, so there is unlikely to have been any bias introduced due to imbalance of genders between experimental groups. With the effects of dietary restraint upon eating behaviours are far from clear (Martins et al., 2008; Johnson et al., 2012), future work should attempt to establish if responses to morning feeding interventions differ by comparing individuals with high and low dietary restraint (as opposed to simply excluding those displaying high restraint).

As described in Chapter 7, breakfast consumption (as prescribed in this study) in obese individuals was associated with greater physical activity energy expenditure during the morning but also weight gain over the course of the intervention period. This weight gain could be suggested to be due to the size of the breakfast prescribed, and therefore future studies should aim to establish if a minimum size and/or specific composition of breakfast maintains physical activity levels but does not result in greater weight gain than omission of breakfast in obese individuals. High protein/fibre breakfasts are a promising first avenue for investigation due to their lasting effects on satiety (Hamedani et al., 2009; Leidy et al., 2013; Belza et al., 2013).

9.10 Conclusions

The work in this thesis has established that extended morning fasting is only minimally compensated for at an *ad libitum* lunch in both lean and obese individuals within a laboratory setting. Carbohydrate rich breakfast consumption causes differing hormonal and metabolic responses to a lunchtime meal, with opposing appetite hormone responses that are similar in both lean and obese individuals, with no difference in subjective appetite during the afternoon. In free-living individuals adhering to 6 weeks of morning fasting, physical activity was lower than those consuming breakfast, particularly in the morning. There was limited dietary compensation for the omission of breakfast in lean individuals but no difference in energy intake between the obese intervention groups. Daily extended morning fasting did not lead to significant weight gain in either lean or obese individuals. Markers of cardiovascular health were largely unaffected by either intervention, although there was some limited evidence of reduced free-living glycaemic control as a result of the fasting intervention in lean and differences in insulin response to an OGTT in obese individuals. Chronic adherence to either free-living intervention had little impact upon acute appetite regulation measured in a laboratory setting.

In conclusion, extended morning fasting does not appear to promote greater energy intake than breakfast consumption, but may limit physical activity to some extent. Chronic adherence to an extended morning fasting regimen does not result in clear deterioration of cardiovascular health markers and measures of glycaemic/insulinaemic control, and there does not appear to be evidence for the popular contention that breakfast skipping leads to weight gain.

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Appendices

Appendix 1: Example Breakfast

Example breakfast as used for laboratory studies outlined in chapters 3, 5, 6 and 8:

For an individual with an RMR of 1600 $kcal \cdot d^{-1}$, they would be provided with a breakfast in the quantities specified below:

Braces White Bread: 60 g
Kellogg's Cornflakes: 40 g
Sainsburys Semi-Skimmed Milk: 199 g
Sainsburys Fresh Orange Juice: 157 g
I Can't Believe It's Not Butter: 9 g
Sainsburys Seedless Raspberry Jam: 11 g

This breakfast provides:

93 g Carbohydrate
9.9 g Fat
16.2 g Protein
504 kcal.

Appendix 2: Obese Actiheart Inclusion Criteria

90/22.5 criteria	Week 1		Week 6			
Participant	Days in Use	EI	Days in Use	EI	Mean of both weeks	Acceptable data?
8	7	**725**	5	**657**	691	Y
13	7	**2569**	6	**1633**	2101	Y
17	1	1453	5	**1296**	1375	N-Week 1 only 1 d
22	7	**821**	0		821	N-Week 6 no data
30	6	**1767**	6	**1576**	1671	Y
38	7	**700**	7	**623**	662	Y
43	0		0			N-No data
45	4	**911**	7	**1148**	1030	Y
46	0		3	**1401**	1401	N-Not enough days
47	5	**1220**	3	**1045**	1132	N-Not enough days
48	6	**519**	7	**517**	518	Y
49	6	**1129**	6	**1368**	1248	Y
51	7	**895**	7	**816**	855	Y
52	2	1457	6	**1446**	1452	N-week 1 only 2 d
54	7	**724**	7	**909**	817	Y
55	7	**304**	5	**521**	413	Y
56	1	812	5	**804**	808	N-Week 1 only 1 d
57	5	**317**	5	**524**	420	Y
58	3	**489**	6	**611**	550	N-Week 1 only 3 d
60	7	**1165**	7	**1227**	1196	Y
61	1	**481**	4	**984**	732	N-Week 1 only 1 d
64	6	**2728**	6	**2085**	2406	Y
65	7	**927**	7	**1129**	1028	Y
Mean	**4.7**	**1053.0**	**5.2**	**1062.9**	**1060.4**	9 individuals excluded
SD	**2.6**	**653.0**	**2.0**	**429.1**	**521.3**	

When analysing the Actiheart daily physical activity data it was established that the data was not as robust as that collected for lean individuals. The data was therefore interrogated to establish if there could be some softening of the criteria so as not to substantially diminish the quality of the data obtained but allow more individuals to be included in the analysis. When doing this, the entire dataset was grouped (independent of experimental group allocation) to avoid knowledge of group results when adjusting inclusion criteria. Included on this (the original data inclusion criteria) and the next page (the revised inclusion criteria) are the obese dataset for Actiheart data depending on the processing method (either allowing a valid day of measurement to comprise daily wear time at a minimum of 90% and recovered data at 22.5%, or the revised criteria of 85% and 30%, respectively. As can be seen, there is

very little difference in the mean (within 12 kcal for the group as a whole) and the SD (within 6 kcal). However, this revised criteria reduces the number of participants excluded from 9 to 4 for physical activity data. It should be noted that despite this change in criteria the average wear time of the devices for valid weeks of measurement was 97.3% of the day, with recovered data (as introduced in Chapter 2) only accounting for 4.6% of the day. Therefore these devices even with softened inclusion criteria for daily data quality are still providing a very comprehensive assessment of habitual activity.

85/30 criteria						
	Days in Use	EI	Days in Use	EI	Mean of both weeks	Acceptable data?
8	**7**	**725**	**5**	**657**	691	Y
13	**7**	**2569**	7	**1626**	2097	Y
17	5	**1390**	5	**1296**	1343	Y
22	7	**821**	0		821	N-Week 6 no data
30	6	**1767**	6	**1576**	1671	Y
38	7	**700**	7	**623**	662	Y
43	1		0			N-No data
45	4	**911**	7	**1148**	1030	Y
46	0		7	**1549**	1549	N-Week 1 no data
47	7	**1022**	4	**1133**	1078	Y
48	6	**519**	7	**517**	518	Y
49	6	**1129**	6	**1368**	1248	Y
51	7	**895**	7	**816**	855	Y
52	4	**1627**	6	**1446**	1536	Y
54	7	**724**	7	**909**	817	Y
55	7	**304**	6	**490**	397	Y
56	3	697	6	**764**	731	N-Week 1 only 3 d
57	6	**329**	7	**478**	403	Y
58	4	**586**	6	**611**	598	Y
60	7	**1165**	7	**1227**	1196	Y
61	5	**780**	5	**1020**	900	Y
64	6	**2728**	6	**2085**	2406	Y
65	7	**927**	7	**1129**	1028	Y
Mean	**5.5**	**1063**	**5.7**	**1070**	**1072**	4 individuals excluded
SD	**2.0**	**644.9**	**2.0**	**441.8**	**526.7**	

Appendix 3: Lean Continuous Glucose Meter Data

	BREAKFAST GROUP				FASTING GROUP			
	Week 1		Week 6		Week 1		Week 6	
Overall	Mean	±SD	Mean	±SD	Mean	±SD	Mean	±SD
24 h mean glucose (mmol/l)	**5.2**	0.4	**5.3**	0.4	**5.3**	0.4	**5.3**	0.4
24 h peak glucose (mmol/l)	**8.2**	1.3	**8.3**	1.2	**8.5**	1.6	**9.4**	3.8
24 h glucose variability (SD)	**0.7**	0.2	**0.7**	0.2	**0.7**	0.2	**0.8**	0.3
24 h glucose variability (CV; %)	**13**	4	**14**	2	**14**	3	**16**	5
Morning								
Waking until 1200 mean glucose (mmol/l)	**5.3**	0.5	**5.5**	0.5	**5.0**	0.4	**5.1**	0.5
Waking until 1200 peak glucose (mmol/l)	**7.4**	1.0	**7.7**	1.3	**6.4**	1.0	**6.7**	1.1
Waking until 1200 glucose variability (SD)	**0.6**	0.2	**0.7**	0.2	**0.5**	0.2	**0.6**	0.2
Waking until 1200 glucose variability (CV; %)	**12**	3	**13**	3	**10**	4	**11**	4
Afternoon/Evening								
1200 until bed mean glucose (mmol/l)	**5.2**	0.4	**5.4**	0.4	**5.3**	0.5	**5.3**	0.4
1200 until bed peak glucose (mmol/l)	**7.7**	1.1	**7.9**	1.3	**8.2**	1.2	**9.1**	3.9
1200 until bed glucose variability (SD)	**0.7**	0.2	**0.7**	0.2	**0.8**	0.2	**0.9**	0.4
1200 until bed glucose variability (CV; %)	**13.39**	3.60	**13.24**	1.29	**14.83**	3.69	**17.16**	7.03
Nocturnal								
Sleeping mean glucose (mmol/l)	**5.1**	0.5	**5.2**	0.4	**5.2**	0.5	**5.2**	0.5
Sleeping peak glucose (mmol/l)	**6.9**	0.9	**6.9**	0.9	**7.0**	1.3	**7.4**	1.6
Sleeping glucose variability (SD)	**0.6**	0.2	**0.6**	0.2	**0.6**	0.2	**0.6**	0.3
Sleeping glucose variability (CV; %)	**12**	5	**12**	4	**12**	4	**12**	4
Wake-Up Time (hh:mm:ss)	**07:21:30**	00:41:03	**07:33:04**	00:42:33	**07:26:34**	00:45:32	**07:15:07**	00:35:31
Bed Time (hh:mm:ss)	**22:46:12**	00:40:17	**22:18:43**	00:49:23	**22:52:15**	00:35:11	**22:35:53**	00:55:45
Sleep Duration (min/night)	**479**	39	**487**	39	**485**	38	**467**	50

For the above outcomes there were no main effects of time or any time x group interaction effects observed (all $p > 0.1$). However, as described in Chapter 4 there were main effects of group observed for morning mean, peak and variability of glucose concentrations (all higher in the breakfast group). There was also greater glucose variability in the afternoon/evening in the fasting group.